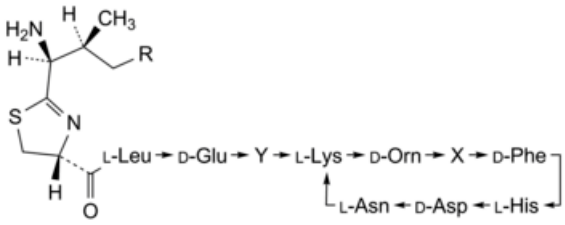
Edition: BP 2025 (Ph. Eur. 11.6 update)

Bacitracin

General Notices

(Ph. Eur. monograph 0465)



Name	Mol. Formula	X	Y	R
Bacitracin A	$C_{66}H_{103}N_{17}O_{16}S$	L-Ile	L-Ile	CH_3
Bacitracin B1	$C_{65}H_{101}N_{17}O_{16}S$	L-Ile	L-Ile	H
Bacitracin B2	$C_{65}H_{101}N_{17}O_{16}S$	L-Val	L-Ile	CH_3
Bacitracin B3	$C_{65}H_{101}N_{17}O_{16}S$	L-Ile	L-Val	CH_3

1405-87-4

Action and use

Polypeptide antibacterial.

Ph Eur

DEFINITION

Mixture of antimicrobial polypeptides produced by certain strains of *Bacillus licheniformis* or *Bacillus subtilis*, the main components being:

— 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin A);

— 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin B1);

— 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin B2);

— 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin B3).

Content

Minimum 60 IU/mg (dried substance).

CHARACTERS

Appearance

White or almost white, hygroscopic powder.

Solubility

Freely soluble in water and in ethanol (96 per cent).

IDENTIFICATION

First identification: B, C.

Second identification: A, C.

A. Thin-layer chromatography (2.2.27).

Test solution Dissolve 10 mg of the substance to be examined in a 3.4 g/L solution of *hydrochloric acid R* and dilute to 1.0 mL with the same solution.

Reference solution Dissolve 10 mg of *bacitracin zinc CRS* in a 3.4 g/L solution of *hydrochloric acid R* and dilute to 1.0 mL with the same solution.

Plate *TLC silica gel plate R*.

Mobile phase *glacial acetic acid R*, *water R*, *butanol R* (14:29:57 *V/V/V*).

Application 10 µL.

Development Over half of the plate.

Drying At 100-105 °C.

Detection Spray with *ninhydrin solution R1* and heat at 110 °C for 5 min.

Results The spots in the chromatogram obtained with the test solution are similar in position, size and colour to the spots in the chromatogram obtained with the reference solution.

B. Composition (see Tests).
C. Ignite 0.2 g. There is no significant yellow-coloured residue at high temperature. Allow to cool. Dissolve the residue in 0.1 mL of *dilute hydrochloric acid R*. Add 5 mL of *water R* and 0.2 mL of *strong sodium hydroxide solution R*. No white precipitate is formed.

TESTS

Solution S

Dissolve 0.25 g in *carbon dioxide-free water R* and dilute to 25 mL with the same solvent.

Appearance of solution

Solution S is clear (2.2.1).

pH (2.2.3)

6.0 to 7.0 for solution S.

Composition

Liquid chromatography (2.2.29): use the normalisation procedure. *Prepare the solutions immediately before use.*

Solution A 40 g/L solution of *sodium edetate R* adjusted to pH 7.0 with *dilute sodium hydroxide solution R*.

Solution B In a volumetric flask, dissolve 54.4 g of *potassium dihydrogen phosphate R* in *water for chromatography R* and dilute to 2000 mL with the same solvent. Adjust to pH 6.0 with a 34.8 g/L solution of *dipotassium hydrogen phosphate R* and filter through a membrane filter (nominal pore size 0.45 µm).

Test solution Dissolve 0.100 g of the substance to be examined in the mobile phase and dilute to 50.0 mL with the mobile phase.

Reference solution (a) Dissolve 20.0 mg of *bacitracin for system suitability CRS* in solution A and dilute to 10.0 mL with the same solution.

Reference solution (b) Dilute 5.0 mL of reference solution (a) to 100.0 mL with solution A. Dilute 1.0 mL of this solution to 10.0 mL with solution A.

Reference solution (c) In order to prepare impurities E, F, G and H *in situ*, heat about 4 mL of reference solution (a) in a water-bath for 30 min. Cool to room temperature.

Column:

— *size*: *l* = 0.15 m, Ø = 4.6 mm;

— *stationary phase*: *base-deactivated end-capped octadecylsilyl silica gel for chromatography R* (3 µm);

— *temperature*: 28 ± 2 °C.

Mobile phase *acetonitrile R*, solution B, *water for chromatography R*, *methanol R* (43:100:300:557 *V/V/V/V*).

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 254 nm.

Injection 100 µL of the test solution and reference solutions (a) and (b).

Run time 3 times the retention time of bacitracin A.

Identification of peaks Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurity M and bacitracins A, B1, B2 and B3 (see Figure 0465.-1).

Relative retention With reference to bacitracin A (retention time = about 20 min): impurity A = about 0.44; impurity B = about 0.52; impurity C = about 0.55; bacitracin B1 = about 0.65; bacitracin B2 = about 0.67; bacitracin B3 = about 0.81; impurity M = about 0.87; impurity N = about 0.90; impurity L = about 0.93; impurity O = about 1.2; impurities P and Q = about 1.3; impurity F = about 1.6; impurity G = about 1.8; impurity H = about 2.1; impurity E = about 2.8.

If necessary, adjust the composition of the mobile phase by changing the amount of organic modifier whilst keeping the ratio constant between methanol and acetonitrile.

System suitability:

— *peak-to-valley ratio*: minimum 1.2, where H_p = height above the baseline of the peak due to bacitracin B2 and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B1 in the chromatogram obtained with reference solution (a);

— *peak-to-valley ratio*: minimum 1.1, where H_p = height above the baseline of the peak due to impurity M and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to bacitracin B3 in the chromatogram obtained with reference solution (a);

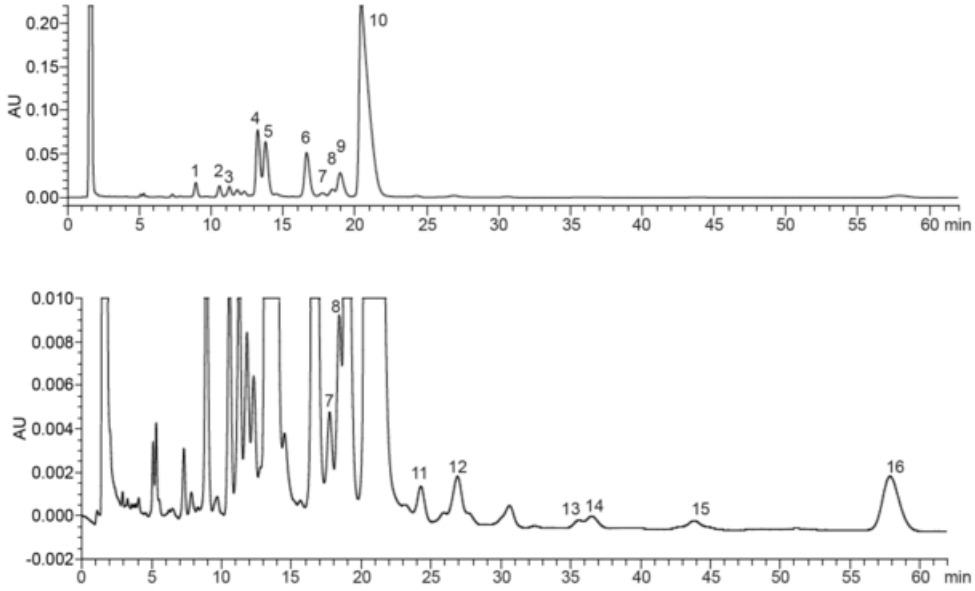
— *signal-to-noise ratio*: minimum 50 for the peak due to bacitracin A in the chromatogram obtained with reference solution (b).

Limits:

— *bacitracin A*: minimum 45.0 per cent;

— *sum of bacitracins A, B1, B2 and B3*: minimum 77.0 per cent;

— *reporting threshold*: 0.25 per cent.



1. impurity A	5. bacitracin B2	9. impurity L	13. impurity F
2. impurity B	6. bacitracin B3	10. bacitracin A	14. impurity G
3. impurity C	7. impurity M	11. impurity O	15. impurity H
4. bacitracin B1	8. impurity N	12. impurities P and Q	16. impurity E

Figure 0465.-1. – *Chromatogram for the test for composition of bacitracin: test solution*

Related substances

Liquid chromatography (2.2.29) as described in the test for composition with the following modifications. Use the normalisation procedure.

Injection Test solution and reference solutions (a), (b) and (c).

Identification of impurities Use the chromatogram obtained with reference solution (a) to identify the peaks due to impurities A, B, C, L, M, N, O, P and Q (see Figure 0465.-1); use the chromatogram obtained with reference solution (c) to identify the peaks due to impurities E, F, G and H (see Figure 0465.-2).

Limits:

— *sum of impurities L and N*: maximum 8.0 per cent;

— *impurity E*: maximum 4.0 per cent;

— *impurity A*: maximum 3.5 per cent;

— *impurities B, M*: for each impurity, maximum 3.0 per cent;

— *impurity C*: maximum 2.5 per cent;

— *sum of impurities O, P and Q*: maximum 2.5 per cent;

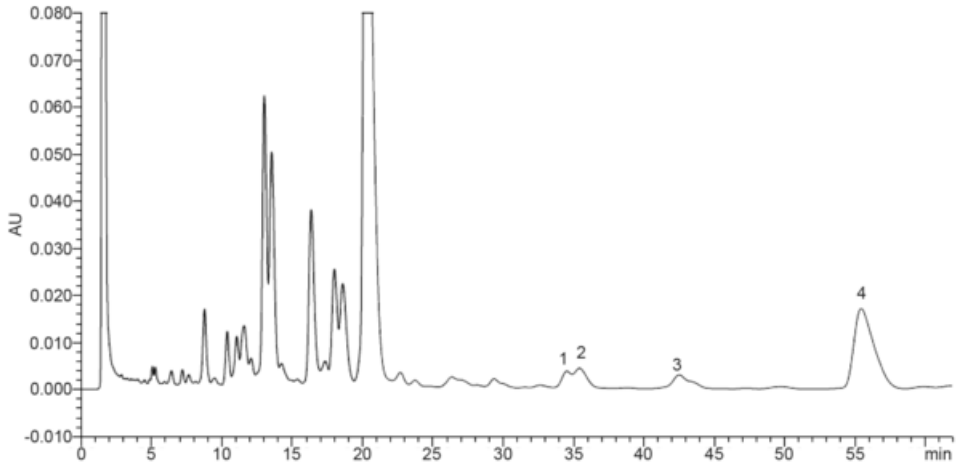
— *sum of impurities F and G*: maximum 2.0 per cent;

— *impurity H*: maximum 1.0 per cent;

— *any other impurity*: for each impurity, maximum 2.0 per cent;

— *total*: maximum 23.0 per cent;

— *reporting threshold*: 0.25 per cent.



1. impurity F 2. impurity G 3. impurity H 4. impurity E

Figure 0465.-2. – *Chromatogram for the test for related substances of bacitracin: reference solution (c)*

Loss on drying (*2.2.32*)

Maximum 5.0 per cent, determined on 1.000 g by drying *in vacuo* at 60 °C at a pressure not exceeding 0.1 kPa for 3 h.

Sulfated ash (*2.4.14*)

Maximum 1.0 per cent, determined on 1.0 g.

ASSAY

Carry out the microbiological assay of antibiotics (*2.7.2*). Use *bacitracin zinc CRS* as the reference substance.

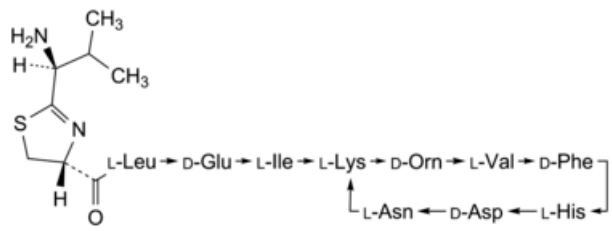
STORAGE

In an airtight container at 2 °C to 8 °C. If the substance is sterile, the container is also sterile and tamper-evident.

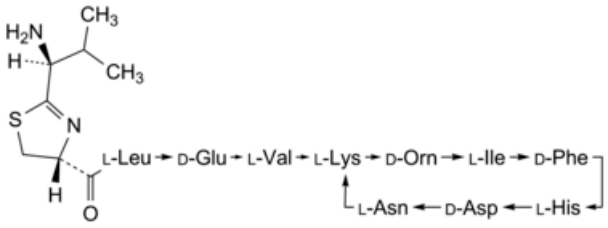
IMPURITIES

Specified impurities *A, B, C, E, F, G, H, L, M, N, O, P, Q.*

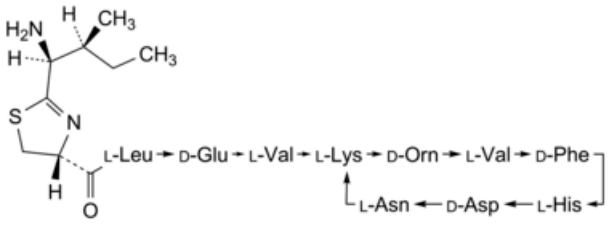
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities. It is therefore not necessary to identify these impurities for demonstration of compliance. See also 5.10. Control of impurities in substances for pharmaceutical use) *D, I, J, K.*



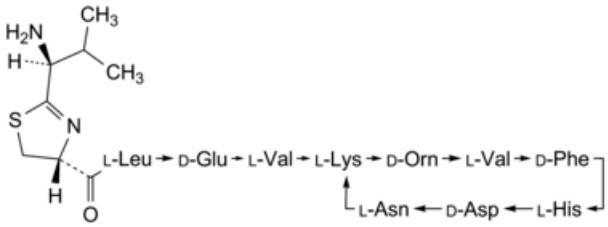
A. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin D1, bacitracin C2),



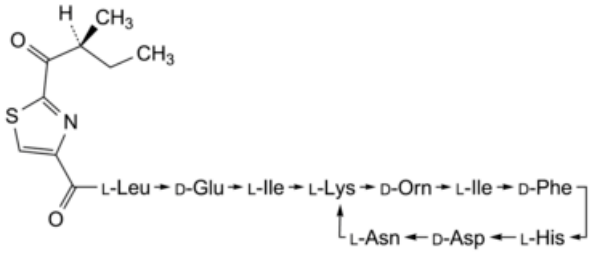
B. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin D2, bacitracin C3),



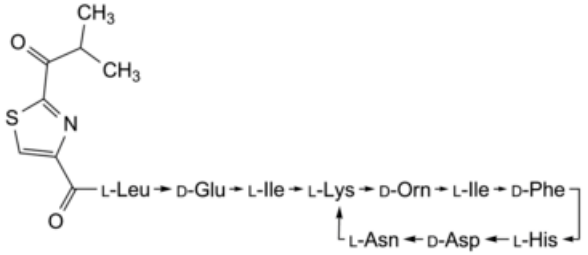
C. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin D3, bacitracin C1a),



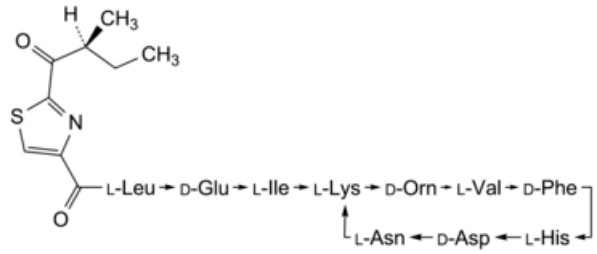
D. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*)-1-amino-2-methylpropyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin E),



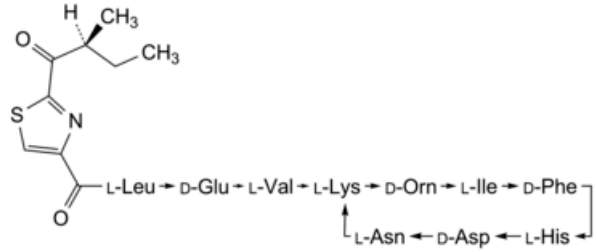
E. 4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin F),



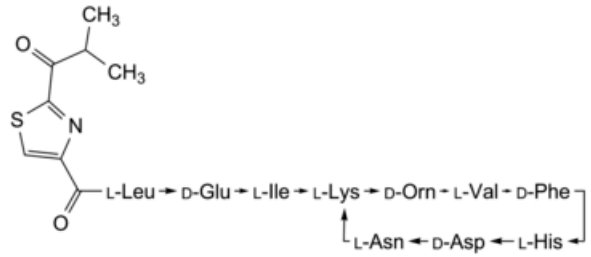
F. 4,10-anhydro[*N*-[[2-(2-methyl-1-oxopropyl)-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin H1),



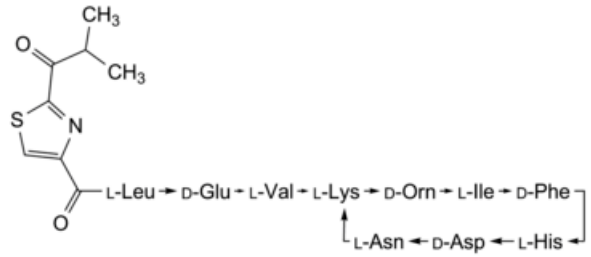
G. 4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin H2),



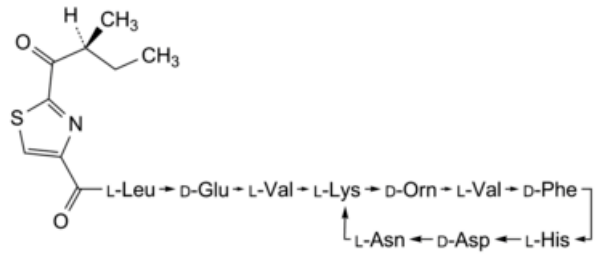
H. 4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin H3),



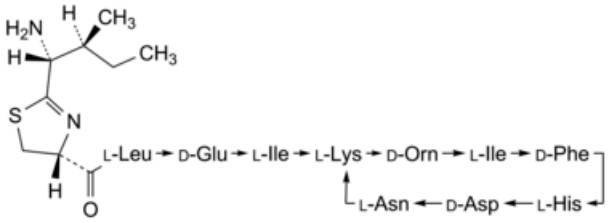
I. 4,10-anhydro[*N*-[[2-(2-methyl-1-oxopropyl)-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin I1),



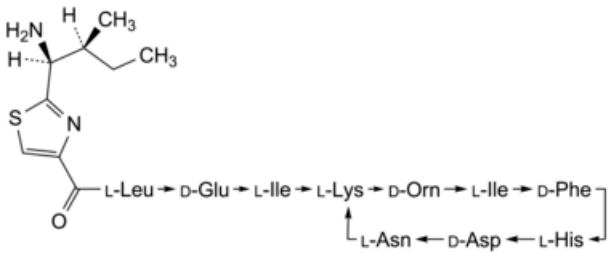
J. 4,10-anhydro[*N*-[[2-(2-methyl-1-oxopropyl)-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin I2),



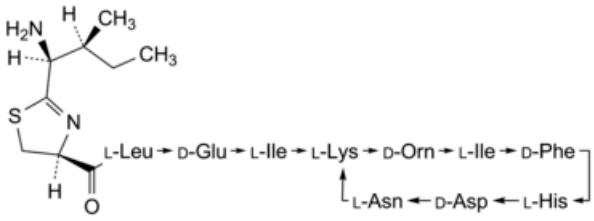
K. 4,10-anhydro[*N*-[[2-[(2*S*)-2-methyl-1-oxobutyl]-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-valyl-L-lysyl-D-ornithyl-L-valyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin I3),



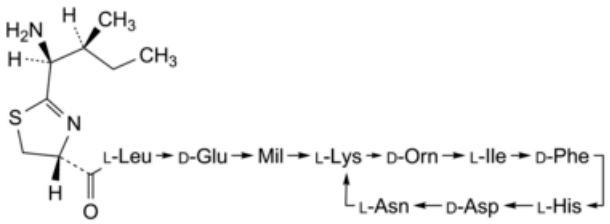
L. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*R*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin X),



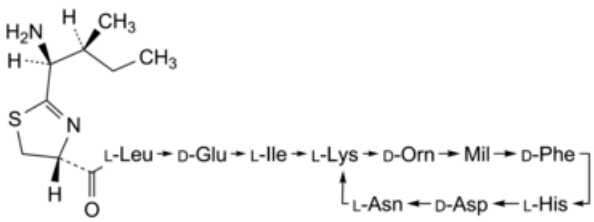
M. 4,10-anhydro[*N*-[[2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin Y),



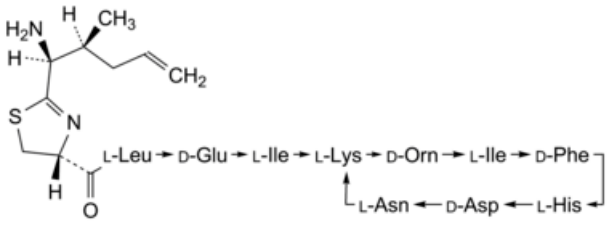
N. 4,10-anhydro[*N*-[[(4*S*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin Z),



O. Mil = 5-methylene-L-isoleucine: 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-5-methylene-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin J1),



P. Mil = 5-methylene-L-isoleucine: 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylbutyl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-5-methylene-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin J2),



Q. 4,10-anhydro[*N*-[[(4*R*)-2-[(1*S*,2*S*)-1-amino-2-methylpent-4-en-1-yl]-4,5-dihydro-1,3-thiazol-4-yl]carbonyl]-L-leucyl-D-α-glutamyl-L-isoleucyl-L-lysyl-D-ornithyl-L-isoleucyl-D-phenylalanyl-L-histidyl-D-α-aspartyl-L-asparagine] (bacitracin J3).

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