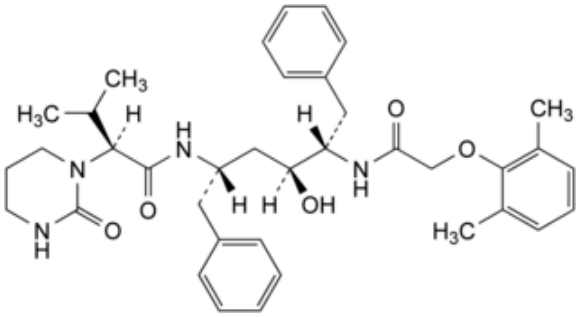
Edition: BP 2025 (Ph. Eur. 11.6 update)

Lopinavir

General Notices

(Ph. Eur. monograph 2615)



$C_{37}H_{48}N_4O_5$ 629 *192725-17-0*

Action and use

Protease inhibitor; antiviral (HIV).

Ph Eur

DEFINITION

(2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-Benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide.

Content

98.0 per cent to 102.0 per cent (anhydrous substance).

CHARACTERS

Appearance

White or yellowish-white, slightly hygroscopic powder.

Solubility

Practically insoluble in water, very soluble in methanol and in methylene chloride.

It shows polymorphism (*5.9*).

IDENTIFICATION

A. Specific optical rotation (see Tests).
B. Infrared absorption spectrophotometry (*2.2.24*).

Comparison *lopinavir CRS*.

If the spectra obtained in the solid state show differences, dissolve the substance to be examined and the reference substance separately in *methanol R*, evaporate to dryness and record new spectra using the residues.

TESTS

Specific optical rotation (*2.2.7*)

-27.0 to -22.0 (anhydrous substance).

Dissolve 0.200 g in *methanol R* and dilute to 25.0 mL with the same solvent.

Related substances

A. Liquid chromatography (*2.2.29*).

Solvent mixture *acetonitrile R1*, *water R* (50:50 *V/V*).

Phosphate buffer solution Dissolve 0.9 g of *dipotassium hydrogen phosphate R* and 2.7 g of *potassium dihydrogen phosphate R* in 900 mL of *water R* and mix well. Adjust to pH 6.0 with *phosphoric acid R*, dilute to 1000 mL with *water R* and filter.

Test solution (a) Dissolve 50.0 mg of the substance to be examined in the solvent mixture and dilute to 100.0 mL with the solvent mixture.

Test solution (b) Dilute 5.0 mL of test solution (a) to 100.0 mL with the solvent mixture.

Reference solution (a) Dissolve 50.0 mg of *lopinavir CRS* in the solvent mixture and dilute to 100.0 mL with the solvent mixture. Dilute 5.0 mL of the solution to 100.0 mL with the solvent mixture.

Reference solution (b) Dilute 5.0 mL of test solution (b) to 250.0 mL with the solvent mixture.

Reference solution (c) Dissolve 2.5 mg of *lopinavir for system suitability CRS* (containing impurities A, B, C, F, G, I, N, Q, R, S and T) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Reference solution (d) Dissolve 2.5 mg of *lopinavir for peak identification CRS* (containing impurities D and O) in the solvent mixture and dilute to 5.0 mL with the solvent mixture.

Column:

— *size*: *l* = 0.25 m, *Ø* = 4.6 mm;

— *stationary phase*: *end-capped octadecylsilyl silica gel for chromatography R* (4 µm);

— *temperature*: 50 °C.

Mobile phase:

— *mobile phase A*: *acetonitrile R1*, phosphate buffer solution (45:55 *V/V*);

— *mobile phase B*: phosphate buffer solution, *acetonitrile R1* (25:75 *V/V*);

Time (min)	Mobile phase A (per cent *V/V*)	Mobile phase B (per cent *V/V*)
0 - 60	100	0
60 - 61	100 → 0	0 → 100
61 - 81	0	100

Flow rate 1.0 mL/min.

Detection Spectrophotometer at 215 nm.

Injection 20 µL of test solution (a) and reference solutions (b), (c) and (d).

Identification of impurities Use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities A, B, C, F, G, I and N; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity D.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 37 min):
impurity A = about 0.03; impurity B = about 0.07; impurity C = about 0.10; impurity D = about 0.13; impurity F = about 0.59; impurity G = about 0.62; impurity I = about 1.1; impurity N = about 1.4.

System suitability Reference solution (c):

— *resolution*: minimum 1.5 between the peaks due to impurities F and G.

Calculation of percentage contents:

— for impurity A, multiply the peak area by the correction factor 1.6;

— for impurity B, multiply the peak area by the correction factor 1.3;

— for impurity C, multiply the peak area by the correction factor 1.5;

— for impurity D, multiply the peak area by the correction factor 1.3;

— for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:

— *impurities B, I*: for each impurity, maximum 0.2 per cent;

— *impurities A, C, D, F, G*: for each impurity, maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *reporting threshold*: 0.05 per cent; disregard any peak eluting after impurity N.

B. Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase Mobile phase A, mobile phase B (30:70 *V/V*).

Run time 8.3 times the retention time of lopinavir.

Identification of impurities Use the chromatogram supplied with *lopinavir for system suitability CRS* and the chromatogram obtained with reference solution (c) to identify the peaks due to impurities Q, R, S and T; use the chromatogram supplied with *lopinavir for peak identification CRS* and the chromatogram obtained with reference solution (d) to identify the peak due to impurity O.

Relative retention r (not r_G) with reference to lopinavir (retention time = about 6 min):

impurity N = about 1.4; impurity O = about 1.5; impurity Q = about 4.4; impurity R = about 6.0; impurity S = about 7.1; impurity T = about 8.5.

System suitability Reference solution (c):

— *resolution*: minimum 3.0 between the peaks due to impurities S and T.

Calculation of percentage contents:

— for impurity O, multiply the peak area by the correction factor 1.3;

— for impurity Q, multiply the peak area by the correction factor 0.7;

— for each impurity, use the concentration of lopinavir in reference solution (b).

Limits:

— *impurities O, Q, R, T*: for each impurity, maximum 0.15 per cent;

— *unspecified impurities*: for each impurity, maximum 0.10 per cent;

— *reporting threshold*: 0.05 per cent; disregard any peak eluting before and including impurity N;

— *total of all impurities eluting before and including impurity N in test A and after impurity N in test B*: maximum 0.7 per cent.

Water

(*2.5.12*): maximum 4.4 per cent, determined on 0.250 g.

Sulfated ash (*2.4.14*)

Maximum 0.2 per cent, determined on 1.0 g.

ASSAY

Liquid chromatography (*2.2.29*) as described in test A for related substances with the following modifications.

Mobile phase Mobile phase A.

Injection Test solution (b) and reference solution (a).

Run time 1.6 times the retention time of lopinavir.

Calculate the percentage content of $C_{37}H_{48}N_4O_5$ taking into account the assigned content of *lopinavir CRS*.

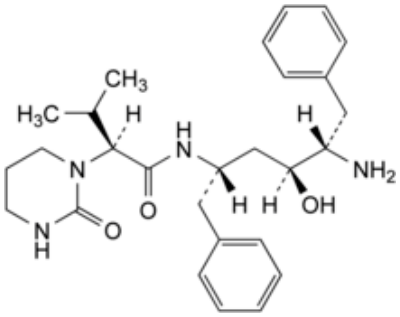
STORAGE

In an airtight container.

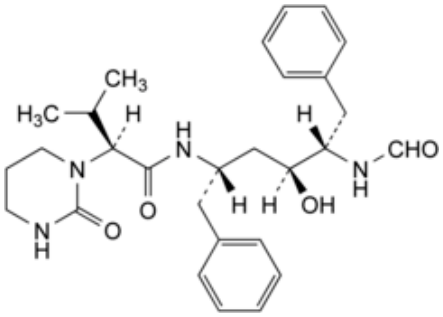
IMPURITIES

Specified impurities A, B, C, D, F, G, I, O, Q, R, T.

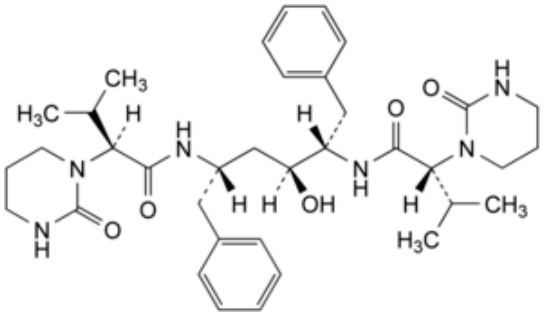
Other detectable impurities (the following substances would, if present at a sufficient level, be detected by one or other of the tests in the monograph. They are limited by the general acceptance criterion for other/unspecified impurities and/or by the general monograph Substances for pharmaceutical use (2034). 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) E, H, J, K, L, M, N, P, S.



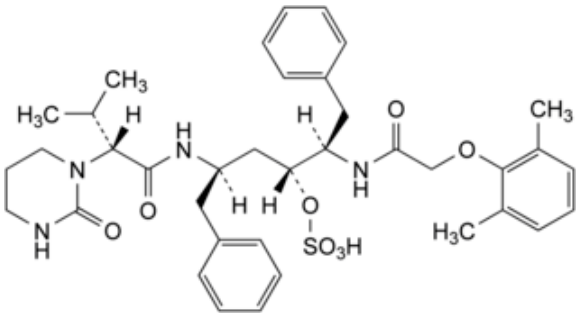
A. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-amino-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



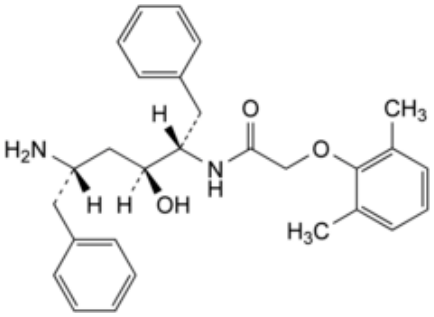
B. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-(formylamino)-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



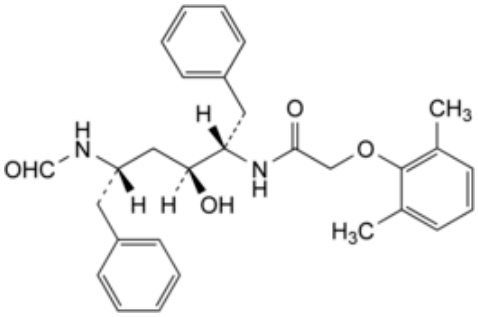
C. (2*R*)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-2-hydroxy-4-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



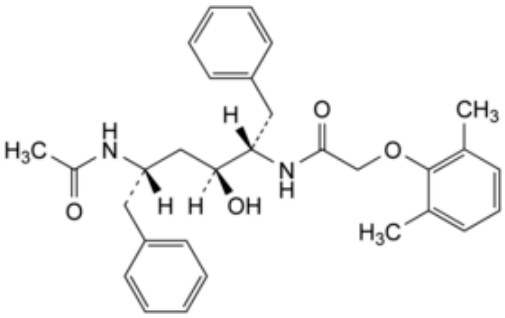
D. (1*R*,3*R*)-1-[(1*R*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*R*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl hydrogen sulfate,



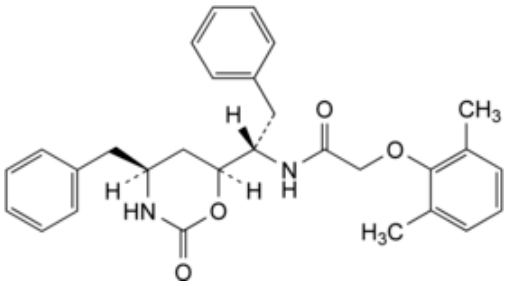
E. *N*-[(1*S*,2*S*,4*S*)-4-amino-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



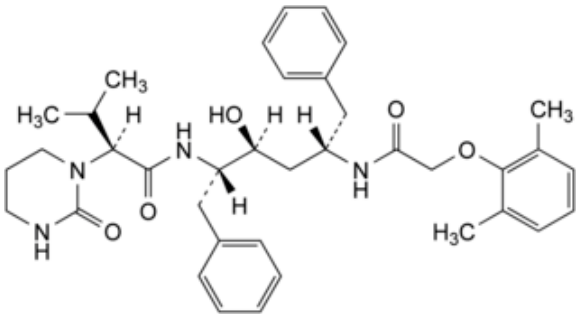
F. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-(formylamino)-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



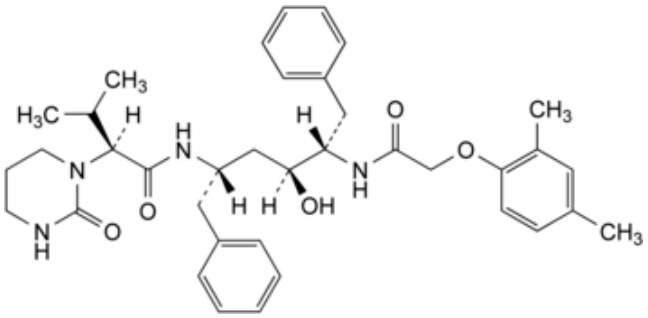
G. *N*-[(1*S*,2*S*,4*S*)-(4-acetylamino)-1-benzyl-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



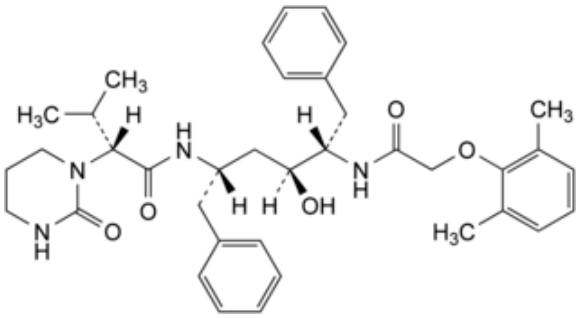
H. *N*-[(1*S*)-1-[(4*S*,6*S*)-4-benzyl-2-oxo-1,3-oxazinan-6-yl]-2-phenylethyl]-2-(2,6-dimethylphenoxy)acetamide,



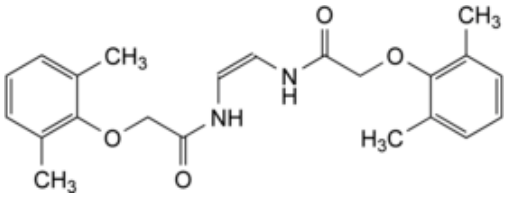
I. (2S)-*N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



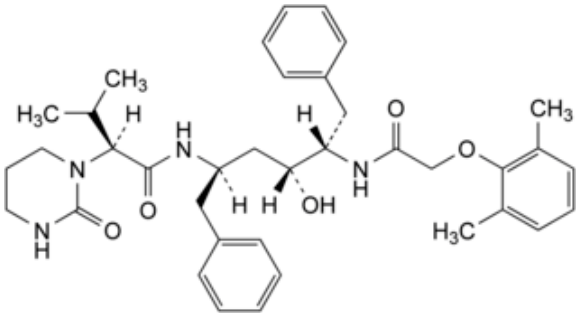
J. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,4-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



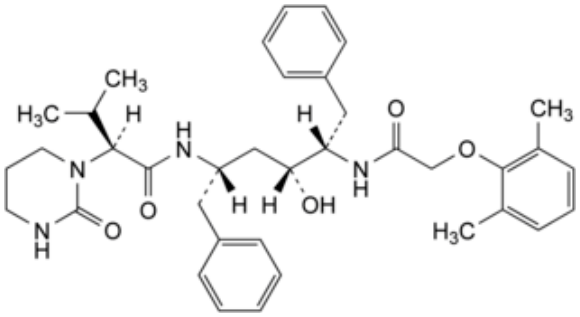
K. (2*R*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



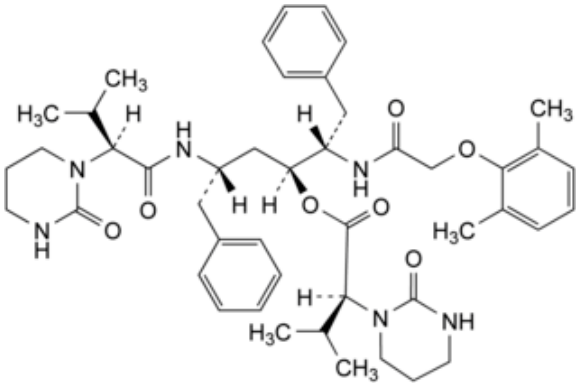
L. *N*,*N*′-(*Z*)-ethene-1,2-diylbis[2-(2,6-dimethylphenoxy)acetamide],



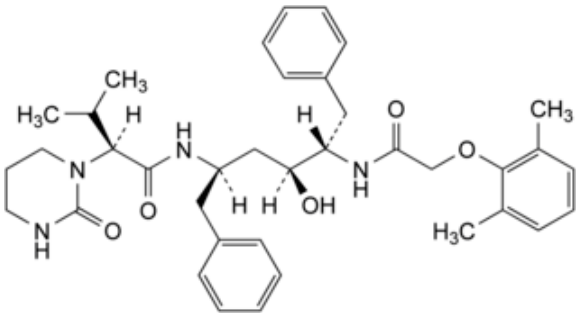
M. (2*S*)-*N*-[(1*R*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



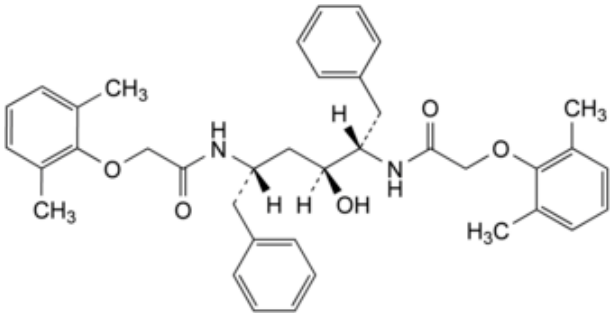
N. (2*S*)-*N*-[(1*S*,3*R*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



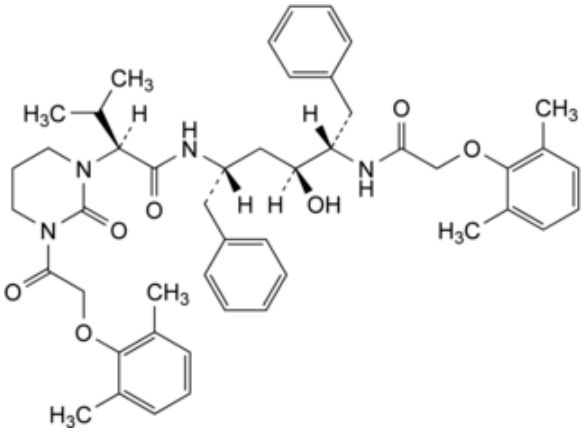
O. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl (2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoate,



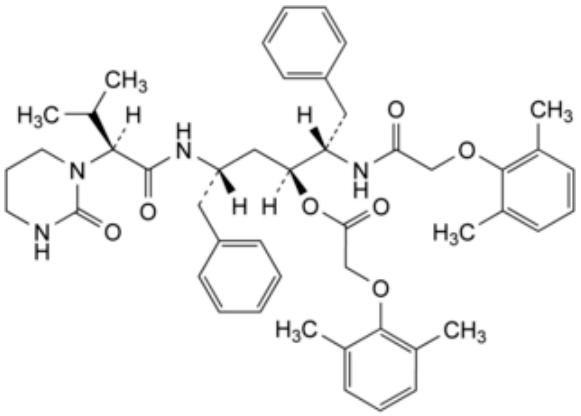
P. (2*S*)-*N*-[(1*R*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanamide,



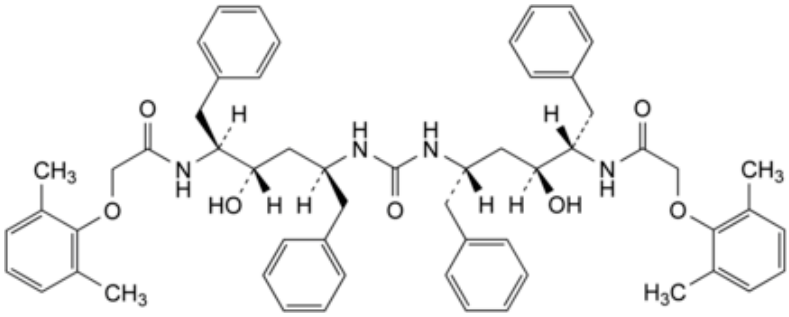
Q. *N*-[(1*S*,2*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-hydroxy-5-phenylpentyl]-2-(2,6-dimethylphenoxy)acetamide,



R. (2*S*)-*N*-[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]-2-[3-[2-(2,6-dimethylphenoxy)acetyl]-2-oxotetrahydropyrimidin-1(2*H*)-yl]-3-methylbutanamide,



S. (1*S*,3*S*)-1-[(1*S*)-1-[[2-(2,6-dimethylphenoxy)acetyl]amino]-2-phenylethyl]-3-[[(2*S*)-3-methyl-2-[2-oxotetrahydropyrimidin-1(2*H*)-yl]butanoyl]amino]-4-phenylbutyl 2-(2,6-dimethylphenoxy)acetate,



T. *N*,*N*′-bis[(1*S*,3*S*,4*S*)-1-benzyl-4-[[2-(2,6-dimethylphenoxy)acetyl]amino]-3-hydroxy-5-phenylpentyl]urea.

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