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Biocatalytical Transformations– VI. [1] The 4-Acetamido-cyclopent-2-ene Carboxylate Route Revisited: Synthesis of (+)- and (-)-Aristeromycin

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Abstract: Enantiomerically pure (+)- as well as (-)-aristeromycin can be synthesized starting from (+)- or (-)-butyl (or hexyl) 4-acetamido-cyclopent-2-ene carboxylate; these carboxylates are easily obtained from their corresponding racemates by hydrolysis with the lipase from *Candida rugosa*.

INTRODUCTION

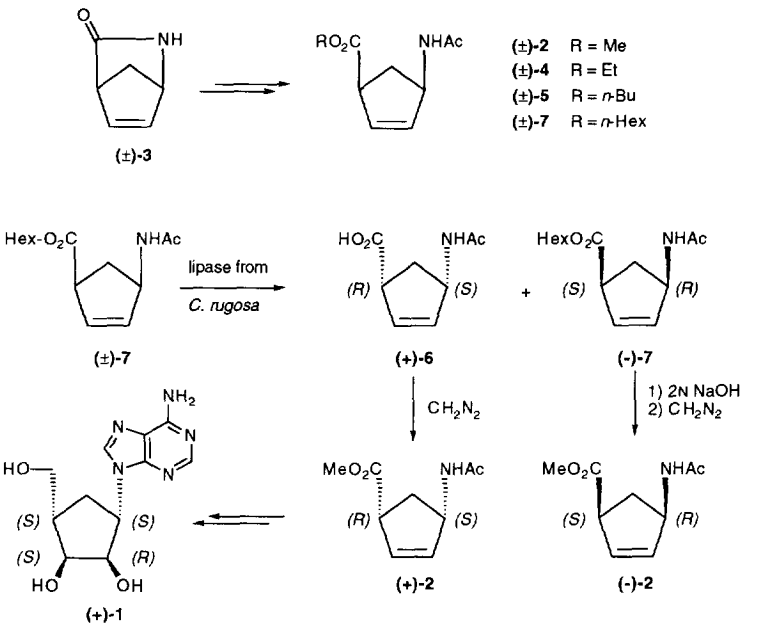
Natural nucleosides and their analogues have been widely studied as potential antitumor, antiviral and fungicidal agents. However, since these nucleosides are substrates for enzymatic degradations a number of modifications have been carried out on both the sugar portion and the heterocycle to avoid or at least to slow down these deactivating processes. Thus, the replacement of the oxygen of the furan ring by a methylene group leads to the synthesis of carbocyclic analogues of nucleosides.[2]

(-)-Aristeromycin, **(-)-1** a secondary metabolite of *Streptomyces citricolor*, [3] is the carbocyclic analogue of adenosine; its cytostatic and antiviral activities have attracted considerable interest and many synthetic and biological studies. In addition, aristeromycin has successfully been used for the synthesis of carbovir [4] showing both potent and selective anti-HIV activity [5] and its derivatives are well known for their antiherpetic activity that resides in a single enantiomer.[6] Thus, many approaches for the enantioselective synthesis of aristeromycin have been devised [7] amongst them chiral pool approaches as well as synthetic schemes using enzyme or microorganism based enantiodifferentiation. The key intermediate of our approach is (±)-methyl (1 *RS*, 4 *SR*) 4-acetamido-cyclopent-2-ene-1-carboxylate **[(±)-2]** which can be obtained in large quantities by the reaction of cyclopentadiene with tosyl-[8] or mesyl [9] cyanide leading to the very common

Vince's lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one (**3**),[8] which is consecutively hydrolysed, esterified and acetylated to yield **2**.[8]

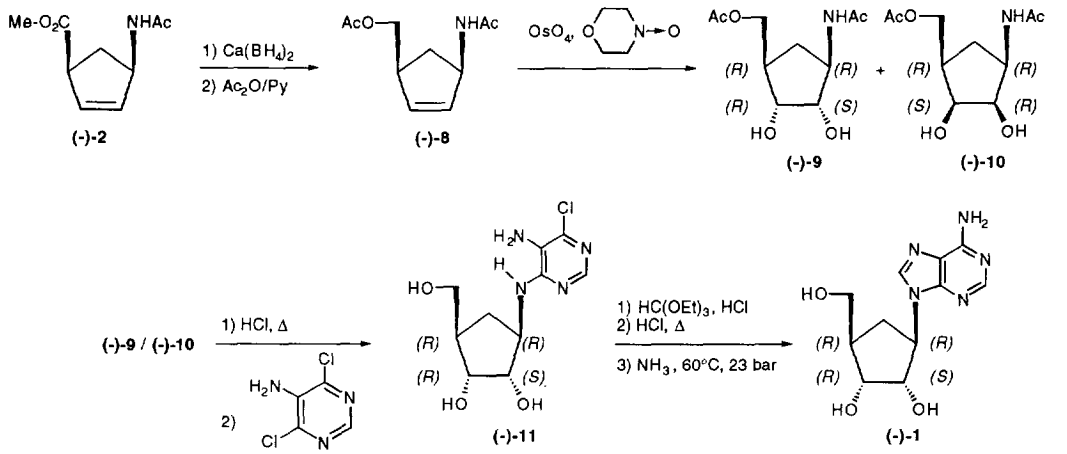
RESULTS AND DISCUSSION

It was claimed [10] that both enantiomers of methyl (1 *RS*, 4 *SR*)-4-acetamido-cyclopent-2-ene carboxylate (**2**) can be accessed by selective enzymatic hydrolysis of **(±)-2** using porcine liver esterase, PLE. It was further claimed that treatment of **(±)-2** with PLE affords after a 50% conversion **(+)-2** with an ee of 87% and **(-)-2** with an ee of 97%, respectively.[10] The ee's of both compounds have been determined by their conversion into the corresponding 4-amino-2,3-dihydroxy-hydroxymethyl-cyclopentanes, well-known precursors for the synthesis of aristeromycin and derivatives.[10]



Unfortunately, in our hands repetition of this procedure using **(±)-2** and PLE did not yield material of the reported [10] enantiomeric excess. Comparison of our data and of data provided in the literature [11] suggested that the enantiomeric excess of the reported ester was only 49% and not 87% as claimed.[11]

Although as an alternative an enantiospecific and enantiocomplementary hydrolysis reaction of the lactam **(±)-3** has been suggested [12] the decision to study the enzymatic hydrolysis of different (1 *RS*, 4 *SR*)-4-acetamido-cyclopent-2-en-1-carboxylates in more detail was taken since these lactam hydrolyses have the need of using either whole cell preparations of microbial strains (*Rhodococcus equi* (NCIB 40213) or *Pseudomonas solanacearum* (NCIB 40249)) or of treating **(±)-3** with special γ-lactamases (from *Pseudomonas fluorescens* or *Aureobacterium sp.*).[12, 13]



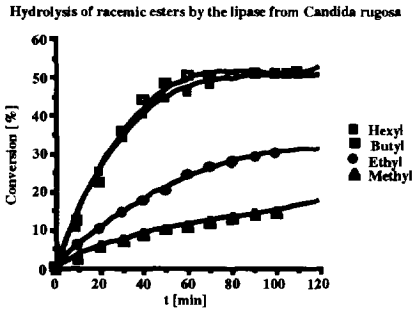
(±)-Methyl (1 *SR*, 4 *RS*) 4-acetamido-cyclopent-2-ene-1-carboxylate [(±)-**2**] has been found to be a suitable substrate for several enzymes,[14] but, unfortunately, all of these enzymes showed unspecific hydrolysis of the ester moiety. The (±) ethyl ester derivative (±)-**4** was substrate for PLE, lipase P, lipase M, lipase CR, lipase B and pancreatin but none of these enzymes afforded an enantioselective hydrolysis.

Table 1: Hydrolysis of racemic butyl ester (±)-**5** by different enzymes

Enzyme	*Temperature [°C]*	*pH*	*Conversion [%]*	*ee [%] of ester*
Esterase R	33	7.0	75.3	0.0
Lipase M	33	7.0	51.0	22.4
Lipase P	25	7.0	65.5	64.8
Lipase B	25	8.0	54.0	87.3
PLE	25	7.0	73.0	91.8
Lipase CR	37	7.0	50.0	≥ 99

The racemic butyl ester (±)-**5** was a non-substrate for penicillinase, leucine aminopeptidase, pronase, lipase A, lipase P, lipase W, acetylcholine esterase, chymotrypsin A_4, papain, lipase N, pancreatin and thermolysin but substrate for the enzymes listed in table 1. It is of interest to note, that the hydrolysis of (±)-**5** with PLE or lipase P afforded (+)-**5** although with rather low ee values whereas its hydrolysis with the other enzymes gave (-)-**5** with fair (lipase M, ee 22%) to excellent (lipase CR, ee ≥ 99%) enantiomeric excess. The hydrolysis with the esterase R from rabbit liver was unspecific at all.

The enzymatic hydrolysis of the racemic hexyl ester (±)-**7** was investigated under a variety of conditions and showed (±)-**7** as a non-substrate for many enzymes whereas upon hydrolysis with the lipase CR (-)-**7** was obtained in enantiomerically pure form. From the corresponding aqueous phase (+)-**2** was obtained after re-esterification with diazomethane. Similarly, (-)-**2** is accessible by saponification of crude (-)-**7** with 2 N NaOH followed by a re-esterification with diazomethane. Thus, enzymatic hydrolysis of either (±)-**5** or (±)-**7** conveniently gives access to both enantiomers of **2**.[15]



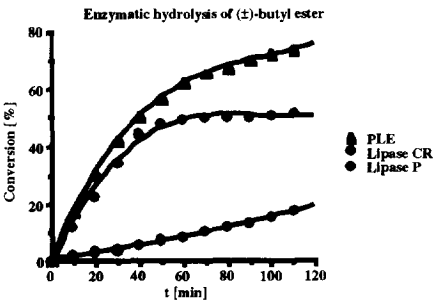


Table 2: Hydrolysis of (±)-**7** by different enzymes

Enzyme	*Temp.[°C]*	*pH*	*Conversion [%]*	*ee [%]*
Lipase M	33	7.0	68.4	29.8
Lipase P	25	7.0	63.4	59.4
Lipase B	25	8.0	51.2	86.4
PLE	25	7.2	83.0	88.1
Lipase CR	37	7.0	50.0	≥ 99

The synthesis of (±)-, (+)- and (-)-aristeromycin **1** is straightforward and follows well-established routes. Thus, the reduction of **2** with calcium borohydride, [8] acetylation with acetic anhydride in the presence of pyridine followed by a *cis*-hydroxylation afforded a 1:1 (by ^{1}H-NMR) mixture of **9**/**10** which without separation upon hydrolysis with 6 N hydrochloric acid followed by the reaction with 5-amino-4,6-dichloro-pyrimidine / triethylamine in *n*-butanol [16,17] gave access to the pyrimidine derivative **11** in good

yields. **11** was treated in succession with triethyl orthoformate, hydrochloric acid and finally with liquid ammonia [8] in a steel autoclave to result in the formation of (±)-, (+)- or (-)-aristeromycin (**1**), respectively.

EXPERIMENTAL

The melting points are uncorrected (*Reichert* hot stage microscope), optical rotations were obtained using a Perkin Elmer 243B polarimeter (1 cm micro-cell), NMR spectra (internal Me_4Si) were recorded using either a Bruker AM250 or a Varian XL300 instrument (δ given in ppm, J in Hz), IR spectra (film or KBr pellet) on a Perkin-Elmer 298 instrument, MS spectra were taken on a Varian-112S instrument; for elemental analysis a Foss-Heraeus Vario EL instrument was used. TLC was performed on silica gel (Merck 5554, detection either by dipping into a solution containing 10% sulfuric acid (400 *ml*), ammonium molybdate (20 g) and cerium(IV) sulfate (20 mg) followed by heating to 150°C or by dipping into a solution of ninhydrin followed by heating to 100°C. Alternatively, the TLC plate was allowed to stand in chlorine atmosphere (developed from $KMnO_4$/aqueous hydrochloric acid) for approx. 5 min, then heated to 100°C and sprayed with a freshly prepared mixture (1:1 *v/v*) of a 0.2 M pyridine solution of 1-phenyl-3-methylpyrazol-5-one and an aqueous 1 N potassium cyanide solution. Moderate warming of the TLC plate afforded red/blue colored spots. HPLC was performed on a Merck-Hitachi L6200A/L4000/D2500 instrument (UV detection at 220 nm) using a 24 cm Serva Chiral=Si/100 (5 μm) β-cylodextrine column and H_2O/MeOH (90:10 *v/v*) as the solvent (1 *ml*/min); the pH-stat equipment was obtained from Büchi.

The enzymes were used as obtained: acetylcholine esterase (*bovine brain*, Sigma, 25°C, pH 8), acetylcholine esterase (*Electrophorus electricus*, Boehringer-Mannheim, Ltd, 37°C, pH 8), acylase I (*Aspergillus melleus*, Fluka, 25°C, pH 7), acylase I (*hog kidney*, Fluka, 25°C, pH 7), AMG 200 L™ (Novo, 25°C, pH 7), Aquazym ultra™ (Novo, 25°C, pH 7), chymotrypsin A_4 (*bovine pancreas*, Boehringer Mannheim Ltd, 25°C, pH 7) esterase R (*rabbit liver*, Sigma, 30°C, pH 7), Fungamyl 800 L™ (Novo, 25°C, pH 7), leucine aminopeptidase (*hog kidney*, Boehringer-Mannheim Ltd, 25°C, pH 7), lipase A6 (*Aspergillus niger*, Amano Ltd, 25°C, pH 7), lipase AY (*Candida rugosa*, Amano, 25°C, pH 7), lipase B (*Candida antarctica* recombinant with *Aspergillus oryzae* immobilised on a macroporous acrylic resin, Novo, 37°C, pH 8), lipase CR (*Candida rugosa*, Boehringer-Mannheim, Ltd, 25°C, pH 7), lipase M10 (*Mucor javanicus*, Fluka, 25°C, pH 7), lipase N (*Rhizopus sp.*, Amano, 25°C, pH 7), lipase P (*porcine pancreas*, Boehringer-Mannheim Ltd, 37°C, pH 7), lipase W (*wheat germ*, Amano, 37°C, pH 7), Lipolase™ (Novo, 25°C, pH 7), pancreatin (*porcine pancreas*, Sigma, 37°C, pH 7.5), papain (*papaya latex*, Sigma, 25°C, pH 7), penicillinase (*Enterobacter cloacae*, Fluka, 25°C, pH 7), PLE (*porcine liver esterase*, Boehringer Mannheim Ltd, 25°C, 37°C, pH 7), pronase (*Streptomyces griseus*, Boehringer-Mannheim Ltd, 25°C, pH 7.5, 8.0, 8.5, 9.0, respectively), subtilisin (*Bacillus lichenformis*, Fluka, 25°C, pH 8), Termanyl 60 L™ (Novo, 25°C, pH 7), thermolysin (*Bacillus thermoproteolyticus*, Fluka, 37°C, pH 7).

(-)-Methyl (1 S, 4 R) 4-acetamido-cyclopent-2-ene-1-carboxylate [(-)-2] and (+)-methyl (1 R, 4 S) 4-acetamido-cyclopent-2-ene-1-carboxylate [(+)-2]. – A suspension of (±)-**7** (5.0 g, 19.8 mmol) and the lipase CR (5 mg, 30 U/mg) in phosphate buffer (pH = 7, 750 *ml*) was stirred at 37°C in the pH-stat equipment.

After addition of a total amount of 100 *ml* of 0.1 N NaOH (corresponding to 50% conversion) the reaction mixture was extracted with ethyl acetate (5 x 200 *ml*). The layers were separated and processed individually. Thus, the combined ethyl acetate extracts were dried over sodium sulfate. The solvent was evaporated, the residue was suspended in 2 N NaOH (125 *ml*), and the mixture was stirred for several hours. After neutralisation with diluted hydrochloric acid the solvent was evaporated under reduced pressure. The residue was suspended in dry methanol (500 *ml*) and an ether solution of diazomethane was added together with one drop of acetic acid. After completion of the reaction the excess of diazomethane was destroyed by adding a few drops of acetic acid, the mixture was washed with a saturated solution of bicarbonate (3 x 20 *ml*) and brine (50 *ml*), dried over magnesium sulfate, the solvent was removed under reduced pressure and the remaining solid subjected to flash chromatography (silica gel, hexane/ethyl acetate 1:1) to yield **(-)-2** (1.45 g, 40%): mp 84-86°C, $[\alpha]_D^{20}$ -86.5 (*c*, 1 $CHCl_3$), ee ≥99% (by HPLC); lit.:[18] mp 89.5-90.5 °C, $[\alpha]_D^{20}$ -84.4 (*c*, 1 methanol).

The aqueous layer was concentrated at reduced pressure and the remaining residue suspended in methanol (200 *ml*). An excess of an ether solution of diazomethane and one drop of acetic acid was added, the mixture was stirred for an additional 30 min and after completion of the reaction a few drops of acetic acid were added in order to destroy the excess of diazomethane. The filtrate was evaporated and water (50 *ml*) and ethyl acetate (500 *ml*) were added. The two phase system was stirred for 20 min, the aqueous layer extracted with ethyl acetate (5 x 50 *ml*) and the combined organic phases were dried over sodium sulfate. The solvent was evaporated and **(+)-2** (1.59 g, 44%) was obtained: mp 83-85°C, $[\alpha]_D^{20}$ +86.7 (*c*, 1.0 $CHCl_3$), R_F (EtOAc/MeOH 5:1) 0.70, IR (KBr): 3300*s*, 3065*w*, 3000*w*, 2955*w*, 2895*w*, 1735*m*, 1625*s*, 1540*s*, 1440*m*, 1380*m*, 1335*w*, 1320*m*, 1300*m*, 1270*m*, 1260*m*, 1250*m*, 1210*s*, 1125*m*, 1085*s*, 1050*m*, 1025*m*, 1010*m*; ^{1}H-NMR (400 MHz, acetone-d_6): 1.77 (*dddd*, *J* = 8.4, 8.4, 11.0, 13.4 Hz, H_A-C(5)), 1.86 (*s*, 3 H, NHAc), 2.52 (*dd*, *J* = 6.8, 13.4 Hz, H_B-C(5), 3.52 (*dddddd*, *J* = 2.3, 2.4, 2.4, 6.8, 8.4, 11.0 Hz, H-C(1)), 3.66 (*s*, 3 H, OMe), 4.92 (*m*, 1 H, H-C(4)), 5.77 (*ddd*, *J* = 2.1, 2.4, 5.6 Hz, H-C(2)), 5.85 (*ddd*, *J* = 2.3, 2.4, 5.6 Hz, H-C(3)); ^{1}H-NMR (250 MHz, $CDCl_3$): 1.80 (*dt*, *J* = 3.8, 13.9 Hz, H_A-C(5)), 1.89 (*s*, 3 H, NHAc), 2.47 (*dt*, *J* = 3.8, 8.5 Hz, H-C(1)), 3.66 (*s*, 3 H, OMe), 4.97 (*dt*, *J* = 3.4, 8.5 Hz, H-C(4)), 5.78 (*m*, 2 H, H-C(2, 3)), 6.18 (*br s*, 1 H, NH); ^{13}C-NMR (125.8 MHz, acetone-d_6): 22.89 (*q*, $COCH_3$), 34.90 (*t*, C(5)), 49.75 (*d*, C(1)), 52.06 (*q*, OMe), 55.29 (*d*, C(4)), 131.69 (*d*, C(2)), 135.27 (*d*, C(3)), 169.18 (*s*, CO), 174.58 (*s*, CO); MS (FAB, glycerol): 185 (M+3, 2%), 184 (M+1, 100%); MS (FAB, glycerol + LiCl): 192 (M+Li+1, 10%), 190 (M+Li, 100%); Anal. Calcd. for $C_9H_{13}NO_3$ (183.21):C, 59.00; H, 7.15; N, 7.65; found: C, 58.85; H, 7.23; N, 7.77.

(±)-(1 RS, 4 SR) Acetic acid 4-acetylamido-cyclopent-2-enyl-methyl ester [(±)-8]. – A suspension of calcium chloride (4.5 g, 40.55 mmol) and sodium borohydride (3.1 g, 81.9 mmol) was stirred in anhydrous THF (85 *ml*) for 3 hours. A solution of **(±)-2** (5.0 g, 27.3 mmol) in anhydrous THF (70 *ml*) was added and stirring was continued for 14 h. The mixture was cooled (5 °C) and ice water (160 *ml*) slowly added. The pH was adjusted to 1.5 by the addition of 6 N hydrochloric acid, the stirring was continued for 1 h and then the solvent was removed under reduced pressure. In succession dry methanol (3 x 50 *ml*) and dry pyridine (3 x

50 *ml*) was distilled off and the remaining residue was suspended in pyridine (37 *ml*). The filtrate and the pyridine washings (2 x 10 *ml*) were combined and acetic anhydride (37 *ml*) was added, the cooling bath was removed and stirring continued for another 12 h. The solvents were removed under reduced pressure and the residue subjected to chromatography (silica gel, ethyl acetate) to afford **(±)-8** (5.17 g, 96%); R_F = 0.77 (ethyl acetate / methanol 5:1), mp 62.5 °C; lit.:[8] 62-63°C; IR (NaCl, film): 3290*s*, 3050*m*, 2975*m*, 2950*s*, 2935*s*, 2890*m*, 2865*m* 1735*s*, 1630*s*, 1530*s*, 1440*s*, 1370*s*, 1295*s*, 1260*s*, 1250*s*, 1240*s*, 1225*s*, 1140*w*, 1125*w*, 1035*s*, 975*w*, 960*w*, 910*w*, 925*w*, 755*s*, 710*s*; ^{1}H-NMR (250 MHz, acetone-d_6): 1.35 (*m*, 1 H, H_A-C(4a)), 1.87 (*s*, 3 H, Me), 2.05 (*s*, 3 H, Me), 2.50 (*m*, 1 H, H_B-C(4a)), 2.95 (*m*, 1 H, H-C(1)), 4.00 (*d*, *J* = 6.5 Hz, 2 H, CH_2O), 4.90 (*m*, 1 H, H-C(4)), 5.58 (*m*, 2 H, H-C(2,3)), 7.20 (*br. s*, 1 H, NH); ^{13}C-NMR (62.89 MHz, acetone-d_6): 171.00 (*s*, CO), 169.45 (*s*, CO), 134.57 (*d*, C(2)), 134.21 (*d*, C(3)), 68.10 (*t*, OCH_2), 54.54 (*d*, C(4)), 44.78 (*d*, C(1)), 35.41 (*t*, C(4a)), 22.92 (*q*, $COCH_3$), 20.75 (*q*, $COCH_3$); MS (FAB, glycerol): 199 (12.0%), 198 (99.0%), 139 (9.5%), 138 (15.0%), 94 (17.0%), 93 (5.2%), 79 (100%), 60 (53.0%), 43 (28.0%); MS (FAB, glycerol + LiCl): 205 (11.5%), 294 (100%), 203 (8.7%), 79 (26.0%), 60 (11.0%), 43 (17.0%); Anal. Calcd. for $C_{10}H_{15}NO_3$ (197.24): C, 60.90; H, 7.67; N, 7.10; found: C, 61.09; H, 7.83; N, 7,24.

(+)-(1* R, *4* S*) Acetic acid 4-acetylamido-cyclopent-2-enyl-methyl ester [(+)-8]. – From **(+)-2** (3.0 g, 16.37 mmol) **(+)-8** was obtained (3.0 g, 93 %), mp 79-82°C, $[\alpha]_D^{20}$ = +34.8 (*c*, 1.2 $CHCl_3$); Anal. Calcd. for $C_{10}H_{15}NO_3$ (197.24): C, 60.90; H, 7.67; N, 7.10; found: C, 61.18; H, 7.79; N, 7.19.

(-)-(1* S, *4* R*) Acetic acid 4-acetylamido-cyclopent-2-enyl-methyl ester [(-)-8]. – From **(-)-2** (3.0 g, 16.37 mmol) **(-)-8** was obtained (3.04 g, 94%), mp 79-81°C; $[\alpha]_D^{20}$ = -35.5 (*c*, 1.5 $CHCl_3$); Anal. Calcd. for $C_{10}H_{15}NO_3$ (197.24): C, 60.90; H, 7.67; N, 7.10; found: C, 61.02; H, 7.87; N, 7.29.

(±)-(1* RS, *2* SR, *3* RS, *5* RS*)-3-(5-Amino-6-chloro-pyrimidin-4-yl-amino)-5-hydroxymethyl-cyclopentane-1,2-diol [(±)-11]. – To a solution of **(±)-8** (2.0 g, 10.14 mmol) and N-methylmorpholine-N-oxide (2.75 g, 20.3 mmol) in acetone/water (8:1, 200 *ml*) osmium tetroxide (130 mg, 0.1 mmol) was added and the mixture stirred for 4 h. The mixture was diluted with acetone (150 *ml*), an excess of solid sodium hydrogen sulfite was added in order to destroy the oxidant and the suspension was filtered through Celite. The filtrate was concentrated at reduced pressure to result in a mixture (4.59 g, 97.7%) of the diastereomeric (1 *RS*, 2 *RS*, 3 *SR*, 5 *RS*)- and (1 *RS*, 2 *SR*, 3 *RS*, 5 *RS*) acetic acid 4-acetylamino-2,3-dihydroxy-cyclopentylmethyl esters **(±)-9** and **(±)-10** (1:1 by 300 MHz ^{1}H-NMR) which could not be separated even by repeated chromatography. [Analytical data for the mixture: IR (film): 3381*s*, 2938*s*, 2360*w*, 2154*w*, 1737*s*, 1732*s*, 1716*s*, 1699*s*, 1660*s*, 1651*s*, 1644*s*, 1633*s*, 1563*s*, 1556*s*, 1537*s*, 1435*s*, 1372*s*, 1255*s*, 1128*s*, 1037*s*, 967*s*, 904*s*; ^{13}C-NMR (75.43 MHz, $CDCl_3$): 172.42 (*s*, CO), 171.61 (*s*, CO), 170.90 (*s*, CO), 170.46 (*s*, CO), 78.33 (*d*), 73.22 (*d*), 73.11 (*d*), 72.16 (*d*), 65.48 (*t*), 63.63 (*t*), 56.64 (*d*), 50.02 (*d*), 42.53 (*d*), 39.27 (*d*), 32.55 (*t*), 29.90 (*t*), 23.31 (*q*), 22.94 (*q*), 20.95 (*q*), 20.89 (*q*); MS (FAB, glycerol): 232 (100%), 190 (18.5%), 172 (24.0%), 112 (20.5%), 60 (38.5%), 43 (67.0%); MS (ei, 80 eV, 103°C): 231 (1.9%), 213 (1.1%), 153 (12.5%), 112 (17.2%), 111 (17.2%), 98 (12.8), 94 (68.1%), 86 (21.4%), 85 (21.6%), 70 (18.7%), 69 (21.1%), 60 (100%), 59 (15.4%), 56 (11.9%), 46 (10.3%), 44 (40.7%), 43 (100%), 41 (10.3%)]. The mixture of diastereomers **9/10** (1.8 g, 7.78 mmol) was suspended in 2 N hydrochloric acid (60 *ml*) and stirred for 45 min

at 70 °C. After neutralisation with 1 N NaOH the solvent was removed at reduced pressure and the residue subjected to chromatography (silica gel, ethyl acetate/methanol 1:1) to afford (±)-N-(2,3-dihydroxy-4-hydroxymethyl-cyclopentyl)-acetamide (0.73 g, 49%); mp 114-116 °C (lit.[16] 117-117.5 °C); R_F 0.41 (ethyl acetate/methanol 1:1) 0.41; IR (film): 3330*s*, 3100*m*, 2935*s*, 2875*m*, 1640*bs*, 1560*bs*, 1440*m*, 1375*s*, 1310*m*, 1260*m*, 1115*s*, 1075*s*, 1035*s*, 965*w*; ^{1}H-NMR (300 MHz, CD_3OD): 1.52 (*m*, 1 H, H_A-C(4a)), 1.95 (*s*, 3 H, NHAc), 2.20 (*m*, 1 H, H_B-C(4a)), 3.52 (*m*, 2 H), 3.70 (*m*, 1 H), 3.85 (*m*, 1 H), 4.10 (*m*, 2 H); ^{13}C-NMR (75.43 MHz, CD_3OD): 173.31 (*s*, CO), 80.32, 73.92 (each *s*, C(2,3)), 62.64 (*t*, CH_2O), 55.52 (*d*, C(1)), 42.40 (*d*, C(4)), 31.69 (*t*, C(4a)), 22.60 (*q*, Me). A suspension of this (±)-N-(2,3-dihydroxy-4-hydroxymethyl-cyclopentyl)-acetamide (0.7 g, 3.70 mmol) in 6 N hydrochloric acid (10 *ml*) was heated under reflux overnight. After cooling to room temperature the solution was decolorized with activated charcoal and the filtrate was evaporated under reduced pressure. The residue was dissolved in water (100 *ml*) and treated with ion exchange resin (column, 50 x 3.5 cm, 250 g Amberlite IR 120 H^+). The resin was rinsed with water (100 *ml*) and the intermediate amino alcohol was eluted with 2 N aqueous ammonia. After evaporation of the solvent, the remaining yellow oil (0.45 g) was suspended in dry *n*-butanol (65 *ml*), triethylamine (1.4 *ml*) and 5-amino-4,6-dichloro-pyrimidine (1.25 g, 7.65 mmol) were added and the mixture was heated under reflux for 14 h. The solvent was removed under reduced pressure and the residue purified by chromatography (silica gel, gradient hexane/ethyl acetate 5:1 → 4:1 → ethyl acetate → ethyl acetate/methanol 5:1) to afford **(±)-11** (0.68 g, 81%); R_F 0.29 (ethyl acetate/methanol 5:1), mp 200-202 °C; IR (KBr): 3346*s*, 2911*s*, 1734*w*, 1700*w*, 1684*s*, 1636*s*, 1587*s*, 1500*s*, 1468*s*, 1420*s*, 1390*s*, 1360*s*, 1341*s*, 1322*s*, 1304*s*, 1274*m*, 1249*m*, 1215*m*, 1195*m*, 1127*s*, 1099*s*, 1080*s*, 1040*s*, 1021*s*, 966*m*, 932*s*, 848*m*, 798*m*, 770*m*; ^{1}H-NMR (300 MHz, DMSO-d_6): 1.15 (*m*, 1 H, H_A-C(4a)), 1.97 (*m*, 1 H, H_B-C(4a)), 2.23 (*m*, 1 H, H_A-C(5)), 3.39 (*m*, 1 H, H_B-C(5)), 3.73 (*br. s*, OH), 4.27 (*m*, 1 H, H-C(1)), 4.48 (*d*, J = 3.6 Hz, 1 H), 4.63 (*t*, J = 3.6 Hz, 1 H), 4.49 (*d*, J = 4.6 Hz, 1 H), 6.75 (*d*, J = 6.7 Hz, NH), 7.75 (*s*, 1 H); ^{13}C-NMR (75.43 MHz, DMSO-d_6): 151.90 (*s*, C(4′)), 145.46 (*d*, C(2′)), 136.61 (*s*, C(6′)), 123.30 (*s*, C(5′)), 76.23 (*d*, C(2)), 72.22 (*d*, C(3)), 63.03 (*t*, C(5)), 56.03 (*d*, C(1)), 45.14 (*d*, C(4)), 30.53 (*t*, C(4a)); MS (ei, 80 eV, 150 °C): 274 (31.8%), 182 (14.3%), 171 (47.8%), 170 (100.5%), 169 (14.6%), 157 (15.5%), 155 (16.8%), 147 (13.0%), 146 (39.8%), 145 (38.5%), 144 (100%).

(+)-(1 S, 2 R, 3 S, 5 S) 3-(5-Amino-6-chloro-pyrimidin-4-ylamino)-5-hydroxymethyl-cyclopentane-1,2-diol [(+)-11]. – From **(+)-8** (2.0 g, 10.14 mmol) **(+)-11** (1.0 g, 36%) was obtained; mp 203-205 °C, $[\alpha]_D^{20}$ +27.9 (*c*, 0.7 MeOH); Anal. Calcd. for $C_{10}H_{15}ClN_4O_3$ (274.71): C, 43.72; H, 5.50; Cl, 12.91; N, 20.40; found: C, 43.84; H, 5.63; Cl, 13.01; N, 20.69.

(-)-(1 R, 2 S, 3 R, 5 R)-3-(5-Amino-6-chloro-pyrimidin-4-ylamino)-5-hydroxymethyl-cyclopentane-1,2-diol [(-)-11]. – From **(-)-8** (2.0 g, 10.14 mmol) **(-)-11** (0.92 g, 33 %) was obtained; mp 24-206 °C, $[\alpha]_D^{20}$ -28.4 (*c* 0.8, MeOH); Anal. Calcd. for $C_{10}H_{15}ClN_4O_3$ (274.71): C, 43.72; H, 5.50; Cl, 12.91; N, 20.40; found: C, 43.89; H, 5.68; Cl, 12.79; N, 20.20.

(±)-Aristeromycin [(±)-1]. – A solution of **(±)-11** (0.63 g, 2.29 mmol) was stirred with triethyl orthoformate (15 *ml*) in the presence of conc. hydrochloric acid (0.26 *ml*) in a sealed flask for 12 h. The

brown suspension was filtered under argon and the precipitate [crude (±)-3-(6-chloro-purin-9-yl)-5-hydroxymethyl-cyclopentane-1,2-diol] was washed with dry diethyl ether (2 x 10 *ml*), dried and then transferred into a steel autoclave containing liquid ammonia (25 *ml*). The autoclave was heated (bath temperature 70 °C, internal pressure 23 bar) for one day. The ammonia was evaporated and the remaining solid dissolved in 0.4 N hydrochloric acid (50 *ml*). The solution was purified by ion exchange chromatography (column, 40 x 3.5 cm, 200 g Dowex 50WX8, 20-50 mesh, elution with 2.5 N aqueous ammonia) to afford **(±)-1** (0.42 g, 75%), R_F 0.28 (ethyl acetate/methanol 5:1), mp 254-257 °C (lit.:[19] 255-256°C); IR (KBr): 3378*bs*, 2950*s*, 2362*w*, 2340*w*, 2168*w*, 1694*bs*, 1651*s*, 1589*s*, 1574*s*, 1548*s*, 1519*s*, 1475*s*, 1455*s*, 1416*s*, 1379*s*, 1345*s*, 1218*s*, 1123*s*, 1039*s*, 968*m*, 890*s*, 846*s*, 790*s*; ^{1}H-NMR: (300 MHz, MeOH-d_4): 1.92-2.00 (*m*, 1 H, H_A-C(5)), 1.96 (*s*, 1 H, OH), 2.20-2.29 (*m*, 1 H, H-C(1)), 2.41-2.51 (*m*, 1 H, H_B-C(5)), 3.67-3.72 (*m*, 2 H, $H_{A,B}$-C(6)), 4.05 (*dd*, 1 H, J = 2.9, 5.5 Hz, H-C(2)), 4.52 (*dd*, 1 H, J = 5.5, 8.9 Hz, H-C(3)), 4.83 (*dd*, 1 H, J = 8.7, 10.4 Hz, H-C(4)), 8.17 (*s*, 1 H, H-C(8′)), 8.21 (*s*, 1 H, H-C(2′)); ^{13}C-NMR (75.43 MHz, DMSO-d_6): 155.93 (*s*, C(6′)), 152.02 (*d*, C(2′)), 149.74 (*s*, C(4′)), 140.08 (*d*, C(8′)), 119.27 (*s*, C(5′)), 74.63 (*d*, C(2)), 71.68 (*d*, C(3)), 62.99 (*t*, C(5)), 59.30 (*d*, C(1)), 45.32 (*d*, C(4)), 29.32 (*t*, C(4a)); MS (ei, 80 eV, 222°C): 266 (1.4%, M+1), 265 (9.4%, M), 248 (2.5%), 162 (30.7), 137 (11.3%), 136 (100%), 135 (59.8%), 108 (17.2%), 81 (11.3%), 55 (11.8%), 54 (11.1%), 45 (15.3%), 44 (12.4%), 43 (27.5%), 41 (10.5%).

(+)-Aristeromycin [(+)-1]. – From **(+)-11** (0.63 g, 2.29 mmol) **(+)-1** (0.41 g, 67 %) was obtained; mp 211-215 °C, $[\alpha]_D^{20}$ +50.3 (*c*, 0.3 DMF).

(-)-Aristeromycin [(-)-1]. – From **(-)-11** (0.63 g, 2.29 mmol) **(-)-1** (0.44 g, 72 %) was obtained; mp 212-214 °C, $[\alpha]_D^{20}$ -51.7 (*c*, 0.35 DMF) [Lit. mp 214 [6], 213-215 °C[3], $[\alpha]_D^{20}$ -51.1 (DMF)[6], -52.5 (DMF)[3]].

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