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A FACILE SYNTHESIS OF ASCAMYCIN AND RELATED ANALOGUES

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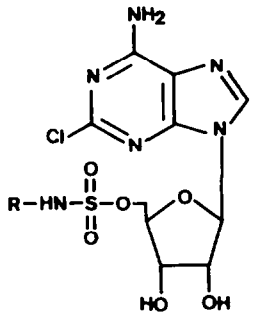
Abstract - The nucleoside antibiotic ascamycin [2-chloro-5'-O-[N-(L-alanyl)sulfamoyl] adenosine (1)] has been synthesized by an improved procedure involving the direct condensation of 2-chloro-2',3'-O-isopropylidene-5'-O-sulfamoyladenosine (3) with Boc-L-Ala-OSu in DMF and in the presence of DBU, followed by removal of the protecting groups. A similar condensation of 3 with Boc-D-Ala-OSu and Boc-Gly-OSu, and subsequent deprotection, yielded the D-Ala and Gly analogues of 1, namely 2-chloro-5'-O-[N-(D-alanyl)sulfamoyl]and 2-chloro-5'-O-[N-(glycyl)sulfamoyl]adenosine [D-ascamycin (14) and (18)]. Similar reactions of 2',3'-O-isopropylidene-5'-O-sulfamoyladenosine, (6) with the three amino acid derivatives above mentioned provided the corresponding adenosine analogues 12, 16 and 20. Several studies directed to demonstrate that the previous protection of the 6-NH_2 group of the adenosine derivatives 3 and 6 is not necessary for the selective aminoacylation of the SO_2NH_2 group are also reported.

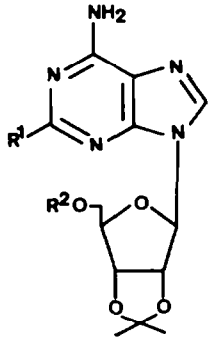
Ascamycin (1) is a new nucleoside antibiotic recently isolated from a fermentation broth of Streptomyces[1]. It shows a remarkably selective antibacterial activity as compared to dealanyl-ascamycin [the antibiotic AT-265[2],(2)[3]]. This selectivity in the case of Xanthomonas citri is due to a dealanylating enzyme present in the bacterial surface which, by removing the alanyl residue, affords dealanylascamycin and facilitates the selective uptake of this nucleoside derivative.

A synthesis of 1 has been reported by Isono et al[4,5] which involves condensation of N^6-benzyloxycarbonyl (Z) or N^6-tert-butyloxycarbonyl(Boc)-2-chloro-2',3'-O-isopropylidene-5'-O-sulfamoyl-



1, Ascamycin, R = L-alanyl

2, Dealanylascamycin, R = H



3, R^1 = Cl ; R^2 = SO_2NH_2

4, R^1 = R^2 = H

5, R^1 = Cl ; R^2 = H

6, R^1 = H ; R^2 = SO_2NH_2

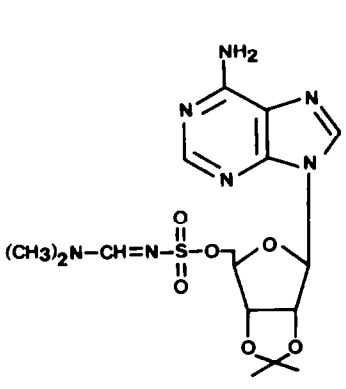
adenosine with Z- or Boc-L-alanylimidazole in DMF using NaH as base, followed by removal of the protecting groups. While the present work was in preparation, a subsequent preliminary communication by the same group[6] reported a related synthesis in which the aminoacylation step was catalyzed by cesium carbonate instead of NaH. According to this paper[6], the use of NaH, employed in the first aminoacylations[4,5], resulted in considerable racemization of the alanine moiety (70% e.e.). The optical yield of the alanyl moiety using cesium carbonate was 88% e.e. No physical constants nor spectral data of any of the synthetic intermediates in the route to 1 were given in any of the above reports[4,5,6].

As part of a project for the synthesis of 5'-O-sulfamoyl nucleosides as nucleotide analogues[7,8,9], we now describe an improved, facile synthesis of Ascamycin which consists on the direct condensation of 3, without previous protection of the 6-NH_2 group, with Boc-L-Ala-OSu[10] using 1.8-diazabicyclo [5, 4, 0] undec-7-ene (DBU) as base, to give 1, after deprotection, in a 76% yield from 3. This shorter procedure, in which no racemization of the L-Ala residue has been detected, has been extended to the synthesis of several amino acid modified analogues of 1, including the D-Ala isomer, as well as to the corresponding adenosine analogues. The interest in the synthesis of 5'-O-[N-(amino-acyl) sulfamoyl]adenosine lies in the biological activity of 5'-O-sulfamoyladenosine itself[11,12,13], which could be liberated after selective microbial deamino-acylation. This biological activity is similar to that of Nucleocidin (4'-fluoro-5'-O-sulfamoyladenosine)[11,14], which shows a broad antibacterial spectrum[15] and is particularly active against trypanosomes[16].

RESULTS AND DISCUSSION

The 2',3'-O-isopropylidene-5'-O-sulfamoyl derivatives of 2-chloroadenosine 3 and adenosine 6, were prepared in 80 and 71% yield, respectively, by reaction of 5 and 4 with bis(tri-n-butyltin) oxide and sulfamoyl chloride following Moffatt's procedure[11].

The key step in the preparation of 1 and analogues is the condensation of the 5'-O-sulfamoyl group of the corresponding 5'-O-sulfamoyladenosine derivative with the amino acid. Therefore, with the aim of attempting a direct condensation of the N^6-unprotected derivatives 3 and 6, we compared the reactivities of the 5'-O-SO_2NH_2 and 6-NH_2 amino groups. In one of these previous experiments, the 5'-O-sulfamoyladenosine derivative 6 was allowed to react with 1 equiv of N,N-dimethylformamide dimethylacetal[17] giving, after 3 h, the corresponding 5'-O-[[N-(dimethylamino)methylene]sulfamoyl] derivative 7 in 85% yield as the only reaction product. The condensation of the 6-amino group of 6 with N,N-dimethylformamide dimethylacetal required 8 h and the use of an excess of the acetal.



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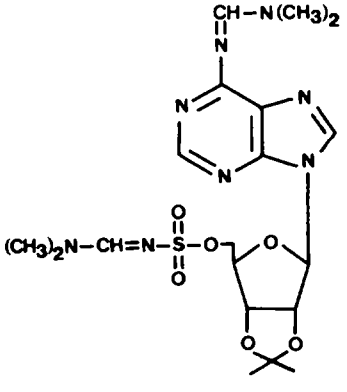

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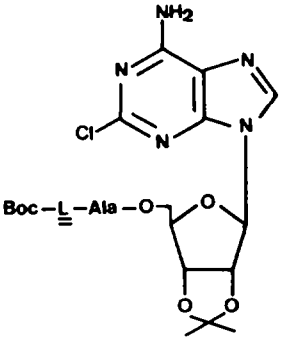

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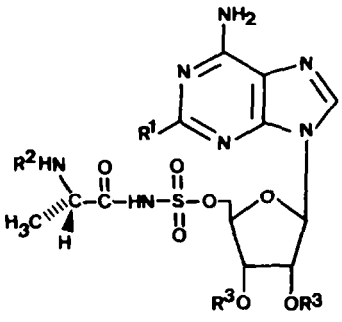
Under these conditions the bis-[(dimethylamino)methylene]substituted nucleoside 8 was obtained. The attachment of the (dimethylamino)methylene function to the 5'-O-sulfamoyl moiety in 7 was deduced by the absence in its ^1H NMR spectrum of the peak at δ 7.55, attributed to the SO_2NH_2 protons of 6 and by the presence of the singlet at δ 7.30 assigned to the 6-NH_2 protons, as

compared to a similar signal δ 7.30 and 7.33 for the same protons of 6 and 5'-O-sulfamoyl adenosine[13] respectively. These experiments suggested us the possible utilization of N^6-unprotected-5'-O-sulfamoyladenosine derivatives in the condensation with amino acids. Further support for this suggestion came out from the reaction of the 2-chloroadenosine derivative 5[19] with 1 equiv of Boc-L-Ala-OSu in DMF and in the presence of Et_3N (2 days), NaH (2h) or DBU (2h). These condensations led to the 5'-O-alanyl ester 9, while no traces of N^6-alanyl derivatives were found. The ^1H NMR spectrum of 9 showed, besides the signals corresponding to the Ala moiety, the presence of the 6-NH_2 protons (δ 7.80) and a downfield shift for the absorption of the 5'-CH_2 protons (δ 4.23) as compared to the same protons (δ 3.70) of 5.

These results, prompted us to achieve the couplings of the N-hydroxysuccinimide esters of Boc-L-Ala, Boc-D-Ala, and Boc-Gly[10] with the 5'-O-sulfamoyladenosine derivatives 3 and 6, without previous protection of the 6-NH_2 group.

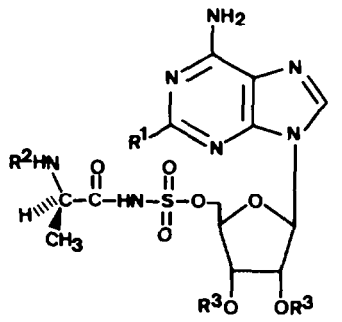
Condensation of 3 with Boc-L-Ala-OSu in DMF using DBU as base gave 2-chloro-2',3'-O-isopropylidene-5'-O-[N-(Boc-L-alanyl)sulfamoyl]adenosine (10) in 80% yield which, after deprotection with trifluoroacetic acid (TFA)/H_2O (5:2), afforded 1[20]. The structures of 10 and 1 were demostrated by analytical and spectroscopic data. Thus, the selective attachment of the alanyl moiety to the sulfamoyl group in 10 was confirmed by the absence in its ^1H NMR spectrum of the singlet assigned to the SO_2NH_2 protons, which in 3 appeared at δ 7.53 and by the presence of a singlet at δ 7.80, assigned to the 6-NH_2 protons, which disappeared by treatment with D_2O, as compared to a similar signal at δ 7.80 for the same protons of 3 and 5. Besides DBU, we have used Et_3N and NaH as bases, in the direct aminoacylation of 3 with Boc-L-Ala-OSu. Although the best results, in terms of reaction time, clean work up and yield (45 and 75% using Et_3N and NaH respectively), were obtained with DBU, the exclusive aminoacylation of the 5'-OSO_2NH_2 amino group was observed in all the cases.



10, R^1=Cl; R^2=Boc; R^3=$C(CH_3)_2$

11, R^1=H; R^2=Boc; R^3=$C(CH_3)_2$

12, R^1=R^2=R^3=H

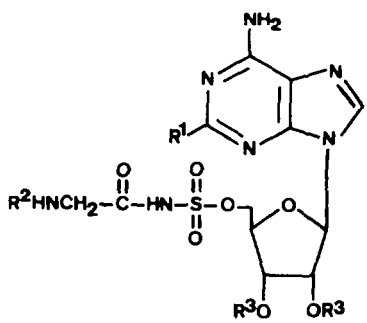


13, R^1=Cl; R^2=Boc; R^3=$C(CH_3)_2$

14, R^1=Cl; R^2=R^3=H (D-Ascamycin)

15, R^1=H; R^2=Boc; R^3=$C(CH_3)_2$

16, R^1=R^2=R^3=H



17, R^1=Cl; R^2=Boc; R^3=$C(CH_3)_2$

18, R^1=Cl; R^2=R^3=H

19, R^1=H; R^2=Boc; R^3=$C(CH_3)_2$

20, R^1=R^2=R^3=H

Similar aminoacylation of 3 with Boc-D-Ala-OSu and Boc-Gly-OSu in the presence of DBU yielded the 5'-O-[N-(Boc-aminoacyl)sulfamoyl]substituted nucleosides 13 and 17 in 80 and 71% yield respectively. Removal of the Boc and isopropylidene protecting groups of 13 and 17 with TFA/H_2O (5:2) gave 2-chloro-5'-O-[N-(D-alanyl)sulfamoyl]adenosine[(14), D-ascamycin] and 2-chloro-5'-O-[(N-glycyl) sulfamoyl]adenosine (18). Finally, a similar series of reactions of 6 with the N-hydroxysuccinimide esters of Boc-L-Ala, Boc-D-Ala, and Boc-Gly gave the corresponding 5'-O-[N-(Boc-aminoacyl)sulfamoyl] adenosine derivatives 11, 15 and 19 in 83, 80 and 74% yield respectively, which upon treatment with TFA/H_2O (5:2) provided the corresponding deprotected compounds, namely 5'-O-[N-(L-alanyl), 5'-O-[N-(D-alanyl) and 5'-O-[N-(glycyl)sulfamoyl]adenosine 12, 16 and 20.

The structures of all these compounds were determined from their 90- (for the protected derivatives 11, 13, 15, 17 and 19) or 360-MHz (for the unprotected compounds 12, 14, 16, 18 and 20) ^1H NMR

spectra. Thus, the attachment of the aminoacyl moiety to the sulfamoyl group in the protected 2-chloroadenosine derivatives 13 and 17 was determined, as in the case of 10, by the presence of singlets at δ 7.80 assigned to the 6-NH_2 protons and by the absence of the signal at 7.53 corresponding to the SO_2NH_2 protons of the starting sulfamoyl nucleoside 3. In a similar way, the structures of compounds 11, 15 and 19 were established as 5'-O-[N-(aminoacyl)sulfamoyl]substituted adenosine derivatives, based on their ^{1}H NMR spectra. They showed, in each case, a singlet at δ 7.28, assigned to the 6-NH_2 protons, as compared to the same protons of 4 and 6 which appeared at δ 7.32 and 7.30, respectively. However, the peak at δ 7.55 corresponding to the SO_2NH_2 protons of 6 did not appear in the spectra of 11, 15 and 19. As in the case of natural Ascamycin[1], the UV spectrum in H_2O of 1, as well as those of the D-Ala and Gly analogs 14 and 18, showed a maximum at 263 nm, while the UV spectra of 12, 16 and 20 showed a maximum at 259 nm. These absorption maxima values correspond to those of the parent 5'-O-sulfamoyl nucleosides 3[12] and 6[11]. Modification of the chromophore by replacement of the 6-NH_2 group by acylamino or sulfonylamino produces a shift ~10 nm of the absorption maximum toward higher wavelength[21]. That no appreciable racemization of the alanyl moiety in compounds 1, 12, 14 and 16 took place, was demonstrated by comparing the 360-MHz ^{1}H NMR spectra of the L-Ala substituted compounds 1 and 12 with those of the corresponding D-Ala isomers 14 and 16. This comparison showed that the ^{1}H NMR spectra of these couples of diastereoisomers (1, 14 and 12, 16) were not superimposable. It also showed that the spectrum of each of those compounds did not show detectable peaks of their corresponding diastereoisomer. Further evidence for these differences came out from the spectra of isomeric mixtures of 1 and 14 or 12 and 16 which in each case, clearly showed two different signals for the alanine CH_3 protons.

In conclusion, the present method for the synthesis of Ascamycin is free of racemization and reduces the number of reaction steps in relation to those previously reported procedures[4,5,6]. This improved method, involving the use of ^{6}N-unprotected sulfamoyl adenosine derivatives, allow to prepare analogs of Ascamycin in high yield.

EXPERIMENTAL

Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 and a Bruker HX-90-E spectrometer using Me_4Si as internal stardard. UV absorption spectra were taken with a Perkin-Elmer 550 SE spectrophotometer. Optical rotations were determined with a Perkin Elmer 141 polarimeter. Analytical TLC was performed on aluminium sheets coated with a 0.2-mm layer of silica gel $60F_{254}$ purchased from Merck and preparative TLC on glass plates coated with a 2-mm layer of silica gel PF_{254} (Merck). Silica gel 60(230-400 mesh) (Merck) was used for column chromatography.

2-Chloro-2',3'-O-isopropylidene-5'-O-sulfamoyladenosine (3). A suspension of 5[19] (4.0 g, 11.4 mmol) in benzene (240 mL) containing hexabutyldistannoxane (13.8 g, 23 mmol) was refluxed under anhydrous conditions for 2 h. The resulting clear solution was cooled to 5°C in a dry box under nitrogen and a solution of sulfamoyl chloride (5.4 g, 47 mmol) in dioxane (80 mL) was added dropwise. After stirring for 30 min the solvent was removed and the residue was extracted with hot hexane. The insoluble residue was treated with dilute methanolic ammonia, evaporated and purified by chromatography on a silica gel column using $CHCl_3$/MeOH(9:1) and then crystallized from acetone-$CHCl_3$ to give 3 (3.9 g, 80%): m.p. 217-220°C (dec);$[\alpha]_D^{20}$ - 16.5° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.30 and 1.50 (2s, 6H, isopropylidene), 4.15(m, 2H, H-5'), 4.36(m, 1H, H-4'), 4.98(dd, 1H, H-3'), 5.32(dd, 1H, H-2'), 6.13(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.53(br s, 2H, SO_2NH_2, D_2O exchangeable), 7.80 (br s, 2H, NH_2-6, D_2O exchangeable), 8.27 (s, 1H, H-8). Anal. Calcd. for $C_{13}H_{17}ClN_6O_6S$: C, 36.70; H, 4.00; Cl, 8.35; N, 19.76; S, 7.53. Found: C, 36.86; H, 4.29; Cl, 8.46; N, 19.39; S, 7.63.

2',3'-O-Isopropylidene-5'-O-sulfamoyladenosine (6). 2',3'-O-Isopropylideneadenosine (4, 3.0 g, 9.7 mmol) was treated with hexabutyldistannoxane (11.5 g, 20 mmol) and sulfamoyl chloride (4.5 g, 39 mmol) as described for the preparation of 3. Purification by column chromatography using $CHCl_3$-MeOH (9:1) and crystallization from acetone-chloroform gave 6 (2.70 g, 71%): m.p. 205-207°C (dec);$[\alpha]_D^{20}$ -23.2° (c 1.0, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.32 and 1.56 (2s, 6H, isopropylidene), 4.20(m, 2H, H-5'),

4.36(m, 1H, H-4'), 5.08(dd, 1H, H-3'), 5.43(dd, 1H, H-2'), 6.22(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.30 (br s, 2 H, NH_2-6, D_2O exchangeable), 7.55 (br s, 2H, SO_2NH_2, D_2O exchangeable), 8.16(s, 1H, H-2), 8.30(s, 1H, H-8). Anal. Calcd. for $C_{13}H_{18}N_6O_6S$: C, 40.40; H, 4.66; N, 21.76; S, 8.39. Found: C, 40.76; H, 4.52; N, 21.53; S, 8.31.

Reaction of 6 with N,N-dimethylformamide dimethyl acetal. To a solution of 6 (0.1g, 0.24 mmol) in DMF (2 mL) cooled at 0°C was added dropwise N,N-dimethylformamide dimethyl acetal (0.03 g, 0.25 mmol) in DMF (1 mL) and the resulting mixture was stirred for 3 h. Then the solvent was evaporated to dryness, the residue was dissolved in $CHCl_3$ (1 mL) and the solution was added, with stirring, to petroleum ether (20 mL). The resulting precipitate was collected and dried over P_2O_5 to give pure 2',3'-O-isopropylidene-5'-O-[[N-(dimethylamino)methylene]sulfamoyl]adenosine (7) (0.11 g, 85%): m.p. 176-178°C; ^{1}H NMR (DMSO-d_6) δ 1.32 and 1.54(2s, 6H, isopropylidene), 2.83 and 3.10 [2s, 6H, $N(CH_3)_2$], 4.10(m, 2H, H-5'), 4.36(m, 1H, H-4'), 5.05(dd, 1H, H-3'), 5.42(dd, 1H, H-2'), 6.20(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.30(br s, 2H, NH_2-6, D_2O exchangeable), 8.06[s, 1H, CH-$N(CH_3)_2$], 8.15(s, 1H, H-2), 8.28(s, 1H, H-8). Anal. Calcd. for $C_{16}H_{23}N_7O_6S$: C, 44.96; H, 5.60; N, 22.22; S, 7.49. Found: C, 44.62; H, 5.46; N, 22.36; S, 7.37.

To a solution of 6 (0.10 g, 0.24 mmol) in DMF (2 mL) was added N,N-dimethylformamide dimethyl acetal (0.10 g, 0.8 mmol) in DMF (2 mL) and the mixture was kept at room temperature for 8 h. Then the solvent was removed and the residue was treated as above to provide 2',3'-O-isopropylidene-N^6-[(dimethylamino)methylene]-5'-O-[[N-(dimethylamino)methylene]sulfamoyl]adenosine (8) (1.09 g, 84%): m.p. 181-183°C; ^{1}H NMR (DMSO-d_6) δ 1.32 and 1.54(2s, 6H, isopropylidene group), 2.83, 3.10, 3.15 and 3.20[4s, 12H, $N(CH_3)_2$], 4.13(m, 2H, H-5'), 4.40(m, 1H, H-4'), 5.07(dd, 1H, H-3'), 5.48(dd, 1H, H-2'), 6.30(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 8.06 and 8.92 [2s, 2H, CH-$N(CH_3)_2$], 8.35(s, 1H, H-2), 8.40(s, 1H, H-8). Anal. Calcd. for $C_{19}H_{28}N_8O_6S$: C, 45.96; H, 5.64; N, 22.58; S, 6.45. Found: C, 45.58; H, 5.80; N, 22.23; S, 6.73.

Reaction of 5 with Boc-L-Ala-OSu. A stirred solution of 5 (0.2 g, 0.57 mmol) and Boc-L-Ala-OSu (0.17 g, 0.58 mmol) in DMF (4 mL) was kept at room temperature in the presence of 1 equiv of the following bases and for the following reaction times: a) triethylamine for two days, b) sodium hydride or DBU for 2 h. Evaporation of the solvent left a residue which was purified by preparative TLC using $CHCl_3$-MeOH (9:1) to afford 5'-O-(Boc-L-alanyl)-2-chloro-2',3'-O-isopropylideneadenosine 9 [a) 0.10 g, 34%, b) 0.17 g, 60%]: m.p. 129-131°C(from MeOH-ether); ^{1}H NMR (DMSO-d_6) δ 1.16 (d, 3H, Ala β CH_3), 1.30 and 1.50(3s, 15H, Boc and isopropylidene groups), 3.82(m, 1H, Ala α CH), 4.23 (m, 3H, H-4' and H-5'), 5.02 (dd, 1H, H-3'), 5.38(dd, 1H, H-2'), 6.13(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.20(d, 1H, Ala NH, D_2O exchangeable), 7.80(s, 2H, NH_2-6, D_2O exchangeable), 8.30(s, 1H, H-8). Anal. Calcd. for $C_{21}H_{29}ClN_6O_7$: C, 49.17; H, 5.66; Cl, 6.93; N, 16.39. Found: C, 48.97; H, 5.84; Cl. 6.57; N, 16.04.

General procedure for the condensation of the 5'-O-sulfamoyl nucleosides 3 or 6 with N-Boc-L-Ala-OSu, N-Boc-D-Ala-OSu and N-Boc-Gly-OSu. A solution of the 5'-O-sulfamoyl nucleoside (1 mmol) and DBU (1 mmol) in DMF (6 mL) was treated with the N-hydroxysuccinimide ester of the Boc-amino acid (1 mmol) and the mixture was stirred at room temperature for 1.5 h. After evaporation of the solvent the residue was purified by column chromatography eluting with $CHCl_3$-MeOH (9:1) to provide the corresponding 5'-O-[N-(Boc-aminoacyl)sulfamoyl]nucleosides which were identified as specified in each case.

2-Chloro-2',3'-O-isopropylidene-5'-O-[N-(Boc-L-alanyl)sulfamoyl]adenosine (10). 80% Yield: m.p. > 250°C (from MeOH-ether)$[\alpha]_D^{20}$-23° (c 0.5 MeOH); ^{1}H NMR (DMSO-d_6) δ 1.16(d, 3H, Ala β CH_3); 1.32 and 1.50(2s, 6H, isopropylidene group), 1.36(s, 9H, Boc), 3.68(m, 1H, Ala α CH), 4.02(m, 2H, H-5'), 4.40 (m, 1H, H-4'), 4.93(dd, 1H, H-3'), 5.25(dd, 1H, H-2'), 6.08(d, 1H, H-1', $J_{1',2'}$= 2.5 Hz), 6.33(d, 1H, Ala NH, D_2O exchangeable), 7.80(s, 2H, NH_2-6, D_2O exchangeable), 8.40(s, 1H, H-8). Anal. Calcd. for $C_{21}H_{30}ClN_7O_9S$: C, 42.60; H, 5.07; Cl, 6.00; N, 16.56; S, 5.40. Found: C, 42.37; H, 4.76; Cl, 6.39; N, 16.27; S, 5.45.

2',3'-O-Isopropylidene-5'-O-[N-(Boc-L-alanyl)sulfamoyl]adenosine (11). 83% Yield: m.p. 211-213°C (dec) (from MeOH-ether); $[\alpha]_D^{20}$- 32.8° (c 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.16(d, 3H, Ala β CH_3), 1.30 and 1.55(2s, 6H, isopropylidene group), 1.34(s, 9H, Boc), 3.68(m, 1H, Ala α CH), 4.00(m, 2H, H-5'), 4.36(m, 1H, H-4'), 5.00(dd, 1H, H-3'), 5.32(dd, 1H, H-2'), 6.16(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.28

(s, 2H, NH_2-6, D_2O exchangeable), 8.14(s, 1H, H-2), 8.36(s, 1H, H-8). Anal. Calcd. for $C_{21}H_{31}N_7O_9S$: C, 45.24; H, 5.56; N, 17.60; S, 5.75. Found: C, 44.88; H, 5.27; N, 17.35; S, 5.46.

2-Chloro-2',3'-O-isopropylidene-5'-O-[N-(Boc-D-alanyl)sulfamoyl]adenosine (13). 80% Yield: m.p.> 250°C (from MeOH-ether); $[\alpha]_D^{20}$- 15.6° (c 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.18(d, 3H, Ala β CH_3); 1.32 and 1.50 (2s, 6H, isopropylidene group), 1.36(s, 9H, Boc), 3.68(m, 1H, Ala α CH), 4.00(m, 2H, H-5'), 4.40(m, 1H, H-4'), 4.95(dd, 1H, H-3'), 5.25(dd, 1H, H-2'), 6.10(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.80(s, 2H, NH_2-6, D_2O exchangeable), 8.40(s, 1H, H-8). Anal. Calcd. for $C_{21}H_{30}ClN_7O_9S$: C, 42.60; H, 5.07; Cl, 6.00; N, 16.56; S, 5.40. Found: C, 42.41; H, 4.78; Cl, 6.37; N, 16.42; S, 5.43.

2',3'-O-Isopropylidene-5'-O-[N-(Boc-D-alanyl)sulfamoyl]adenosine (15). 80% Yield: m.p. 209-210°C (dec) (from MeOH-ether); $[\alpha]_D^{20}$- 4.3° (c 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.15(d, 3H, Ala β CH_3), 1.30 and 1.55(2s, 6H, isopropylidene group), 1.33(s, 9H, Boc), 3.68(m, 1H, Ala α CH), 4.00(m, 2H, H-5'), 4.36(m, 1H, H-4'), 5.00(dd, 1H, H-3'), 5.35(dd, 1H, H-2'), 6.16(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.28 (s, 2H, NH_2-6, D_2O exchangeable), 8.12(s, 1H, H-2), 8.35(s, 1H, H-8). Anal. Caldc. for $C_{21}H_{31}N_7O_9S$: C, 45.24; H, 5.56; N, 17.60; S, 5.75. Found: C, 44.87; H, 5.21; N, 17.39; S, 5.72.

2-Chloro-2',3'-O-isopropylidene-5'-O-[N-(Boc-glycyl)sulfamoyl]adenosine (17). 71% Yield: m.p.> 250°C (from MeOH-ether); $[\alpha]_D^{20}$- 10.8° (c, 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.32 and 1.50 (2s, 6H, isopropylidene group), 1.35(s, 9H, Boc), 3.40(d, 2H,Gly CH_2), 4.00(m, 2H, H-5'), 4.40(m, 1H, H-4'), 4.95(dd, 1H, H-3'), 5.25(dd, 1H, H-2'), 6.10(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.80(s, 2H, NH_2-6, D_2O exchangeable), 8.40(s, 1H, H-8). Anal. Calcd. for $C_{20}H_{28}ClN_7O_9S$: C, 41.56; H, 4.84; Cl, 6.15; N, 16.97; S, 5.54. Found: C, 41.18; H, 4.46; Cl, 6.38; N, 16.71; S, 5.45.

2',3'-O-Isopropylidene-5'-O-[N-(Boc-glycyl)sulfamoyl]adenosine (19). 74% Yield: m.p. 208-210 °C(dec) (from MeOH-ether); $[\alpha]_D^{20}$- 16.1° (c 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 1.30 and 1.50 (2s, 6H, isopropylidene group), 1.34(s, 9H, Boc), 3.37(d, 2H, Gly CH_2), 4.00(m, 2H, H-5'), 4.35(m, 1H, H-4'), 5.00(dd, 1H, H-3'), 5.35(dd, 1H, H-2'), 6.15(d, 1H, H-1', $J_{1',2'}$= 2 Hz), 7.28(s, 2H, NH_2-6, D_2O exchangeable), 8.13(s, 1H, H-2), 8.35(s, 1H, H-8). Anal. Calcd. for $C_{20}H_{29}N_7O_9S$: C, 44.20; H, 5.34; N, 18.05; S, 5.89. Found: C, 43.89; H, 5.00; N, 17.78; S, 5.76.

Deprotection reactions. General procedure. A suspension of the corresponding 2',3'-O-isopropylidene-5'-O-[N-(Boc-aminoacyl)sulfamoyl]nucleoside (0.5 mmol) in a mixture of TFA/H_2O (5:2) (4 mL) was stirred at room temperature for 2 h. The solvent was evaporated and the residue was co-evaporated three times with EtOH and purified by column chromatography, eluting with $CHCl_3$-MeOH (2:1) for compounds 1, 12, 14 and 16, and with $CHCl_3$-MeOH (1:1) for compounds 18 and 20.

2-Chloro-5'-O-[N-(L-alanyl)sulfamoyl]adenosine (Ascamycin, 1). 95% Yield: m.p.>270°C (from EtOH-H_2O), lit[1,20] m.p. > 270°C; $[\alpha]_D^{20}$ -3.7°(c 0.5, H_2O), lit[1,20] [α] +2.3°(c 1.0, H_2O); UV λ_{max} (H_2O) 263 nm (ε 12500); lit[1] UV λ_{max} (H_2O) 263 nm (ε 12270); ^{1}H NMR (D_2O, 360 MHz) δ 1.26(d, 3H, Ala β CH_3, J= 7.18 Hz), 3.60(q, 1H, Ala α CH), 4.21(m, 2H, H-5'), 4.25(m, 1H, H-4'), 4.31(t, 1H, H-3'), 4.52(t, 1H, H-2'), 5.83(d, 1H, H-1', $J_{1',2'}$= 4.70 Hz), 8.14(s, 1H, H-8). Anal. Calcd. for $C_{13}H_{18}ClN_7O_7S$: C, 34.55; H, 3.99; Cl, 7.86; N, 21.70; S, 7.08. Found: C, 34.88; H, 4.32; Cl, 7.58; N, 21.46; S, 6.92.

5'-O-[N-(L-Alanyl)sulfamoyl]adenosine (12). 94% Yield: m.p. 215°C (dec) (from EtOH-H_2O); $[\alpha]_D^{20}$- 12.8°(c 0.5, H_2O); UV λ_{max} (H_2O) 259 nm (ε 11300); ^{1}H NMR (DMSO-d_6+D_2O, 360 MHz) δ 1.30(d, 3H, Ala β CH_3, J = 7.14 Hz), 4.05-4.17(m, 4H, H-3', H-4', H-5'), 4.58(t, 1H, H-2'), 5.91(d, 1H, H-1', $J_{1',2'}$= 5.86 Hz), 8.14(s, 1H, H-2), 8.37(s, 1H, H-8). Anal. Calcd. for $C_{13}H_{19}N_7O_7S$: C, 34.51; H, 4.20; N, 21.68; S, 7.08. Found: C, 34.32; H, 4.50; N, 21.53; S, 7.00.

2-Chloro-5'-O-[N-(D-alanyl)sulfamoyl)]adenosine (D-Ascamycin, 14). 95% Yield: m.p.> 250°C (from EtOH-H_2O); $[\alpha]_D^{20}$-11.5°(c 0.5, H_2O); UV λ_{max} (H_2O) 263 nm (ε 13100); ^{1}H NMR (D_2O, 360 MHz) δ 1.27(d, 3H, Ala β CH_3, J= 7.18 Hz), 3.64(q, 1H, Ala α CH), 4.20(m, 2H, H-5'), 4.23(m, 1H, H-4'), 4.31(t, 1H, H-3'), 4.52(t, 1H, H-2'), 5.83(d, 1H, H-1', $H_{1',2'}$= 4.92 Hz), 8.14(s, 1H, H-8). Anal. Calcd. for $C_{13}H_{18}ClN_7O_7S$: C, 34.55; H, 3.99; Cl, 7.86; N, 21.70; S, 7.08. Found: C, 34.43; H, 3.66; Cl, 7.63; N, 21.53; S, 6.82.

5'-O-[N-(D-Alanyl)sulfamoyl]adenosine (16). 93% Yield: m.p. 212°C (dec); $[\alpha]_D^{20}$-18.3°(c 0.5, H_2O); UV λ_{max} (H_2O) 259 nm (ε 13000); ^{1}H NMR (DMSO-d_6 + D_2O, 360 MHz) δ 1.28(d, 3H, Ala β CH_3, J=

7.10 Hz), 4.03-4.18(m, 4H, H-3', H-4', H-5'), 4.59(t, 1H, H-2'), 5.91(d, 1H, H-1', $J_{1',2'}$= 5.87 Hz), 8.14(s, 1H, H-2), 8.37(s, 1H, H-8). Anal. Calcd. for $C_{13}H_{19}N_7O_7S$: C, 34.51; H, 4.20; N, 21.68; S, 7.08. Found: C, 34,21; H, 4.45; N, 21.34; S, 7.41.

2-Chloro-5'-O[N-(Glycyl)sulfamoyl] adenosine (18). 96% Yield: m.p.> 250°C; $[\alpha]_D^{20}$-15.4°(c 0.5, H_2O); UV λ_{max} (H_2O) 263 nm (ε 12200); ^{1}H NMR (DMSO-d_6+D_2O, 360 MHz) δ 3.24(s, 2H, Gly CH_2), 4.05-4.15 (m, 4H, H-3', H-4', H-5'), 4.50(t, 1H, H-2'), 5.83(d, 1H, H-1', $J_{1',2'}$= 5.96 Hz), 8:41(s, 1H, H-8). Anal. Calcd. for $C_{12}H_{16}ClN_7O_7S$: C, 32.88; H, 3.65; Cl, 8.10; N, 22.37; S, 7.31. Found: C, 32.50; H, 4.03; Cl, 8.28; N, 22.68; S, 7.65.

5'-O-[N-(Glycyl)sulfamoyl]adenosine (20). 95% Yield: m.p. 212°C (dec); $[\alpha]_D^{20}$- 22.3° (c 0.5, H_2O); UV λ_{max} (H_2O) 259 nm (ε 11000); ^{1}H NMR (DMSO-d_6+D_2O, 360 MHz) δ 3.22(s, 2H, Gly CH_2), 4.04-4.15(m, 4H, H-3', H-4', H-5'), 4.58(t, 1H, H-2'), 5.90(d, 1H, H-1', $J_{1',2'}$= 5.86 Hz), 8.14(s, 1H, H-2), 8.38(s, 1H, H-8). Anal. Calcd. for $C_{12}H_{17}N_7O_7S$: C, 35.62; H, 3.88; N, 22.37; S, 7.30. Found: C, 35.71; H, 4.25; N, 22.27; S, 7.02.

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