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# Antibacterial 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines

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ABSTRACT

The aminoacyl-tRNA synthetase (aaRS) class of enzymes is a validated target for antimicrobial development. Aminoacyl analogues of 5′-*O*-(*N*-L-aminoacyl)-sulfamoyladenosines are known to be potent inhibitors of aaRS, but whole cell antibacterial activity of these compounds is very limited, and poor penetration into bacteria has been proposed as the main reason for this. Aiming to find derivatives that better penetrate bacteria, we developed a simple and short method to prepare dipeptidyl-derivatives of 5′-*O*-(*N*-L-aminoacyl)-sulfamoyladenosines, and used this method to prepare 18 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines. The antibacterial activity of these derivatives and a number of reference compounds against *S. aureus*, *E. faecalis* and *E. coli* was determined. Several of the new derivatives showed improved antibacterial activity and an altered spectrum of antibacterial activity.



## 1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are the enzymes that catalyze the transfer of amino acids to their cognate tRNA.[1] They play an essential role in protein biosynthesis and are required for both growth and survival of all cells. The aminoacyl-tRNA synthetase class of enzymes is clinically validated as target for antimicrobial drug development, as exemplified by the clinical use of mupirocin, an inhibitor of bacterial IleRS, in the topical treatment of bacterial colonizations.[2]

At the moment, mupirocin is the only aminoacyl-tRNA synthetase inhibitor in clinical use. An inhibitor of LeuRS, AN-2690 (**3**, Fig. 1), is currently in clinical development for the topical treatment of onychomycosis,[3,4] and an inhibitor of IleRS, icofungipen (**4**), reached clinical phase II for the systemic treatment of oropharyngeal candidiasis. While the latter compound showed a good clinical response in this trial, mycologic eradication rates were too low to support further development.[5]

The largest group of aaRS inhibitors (aaRSi) are the reaction-intermediate mimics. These compounds are analogues of aa-AMP (**1**, Fig. 1), which is the natural reaction intermediate of these enzymes. Mupirocin belongs to this group, as do the twenty 5′-*O*-(*N*-L-aminoacyl)-sulfamoyladenosines (aaSA, **2**). The latter are commercially available and show $K_i$ values in the low nanomolar

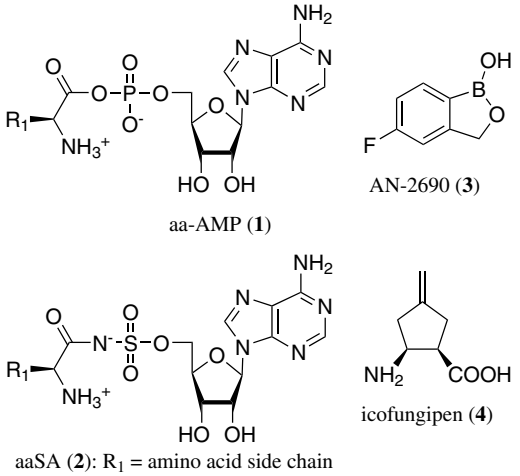


**Figure 1.** Structures of universal aaRS intermediate aa-AMP (**1**), universal aaRS inhibitor aaSA (**2**) and investigational aaRS inhibitors AN-2690 (**3**) and icofungipen (**4**).

*Abbreviations:* aa, amino acid; aaRS, aminoacyl-tRNA synthetases (where aa can be replaced by any amino acid 3-letter code to indicate individual enzymes); aaRSi, aminoacyl-tRNA synthetases inhibitor; aaSA (were aa can be replaced by any amino acid 3-letter code), 5′-*O*-(*N*-aminoacyl)-sulfamoyladenosine; Boc, *tert*-butoxycarbonyl; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; EDCI·HCl, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, *N*-hydroxybenzotriazole; McC, Microcin C; –OSu, *N*-hydroxysuccinimide ester; rt, room temperature; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; THF, tetrahydrofurane.

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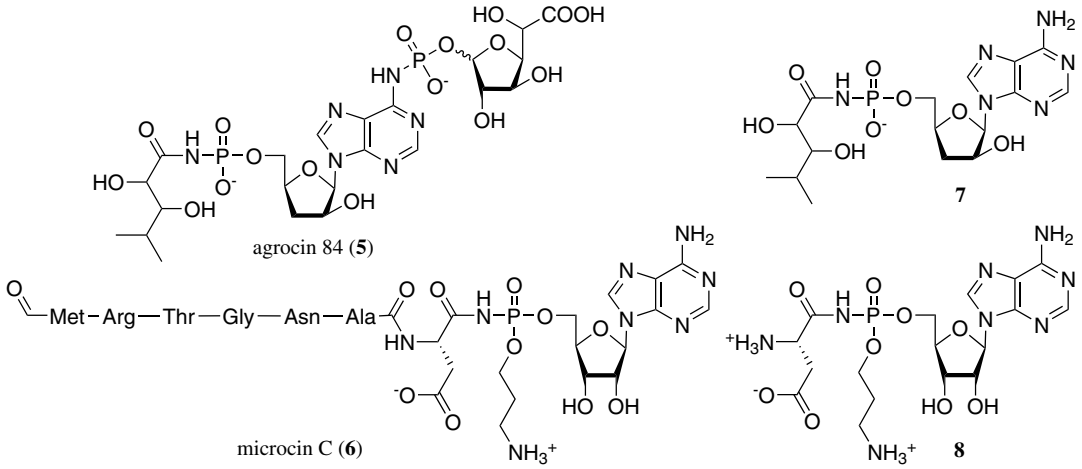



**Figure 2.** Structures of the Trojan Horse antibiotics agrocin 84 (**5**) and microcin C (**6**), and their aaRS inhibiting payloads **7** and **8**, respectively.

range against the corresponding isolated aaRSs.[6–10] Since the aaSA compounds differ only little from the natural reaction intermediates, they do not show noticeable selectivity towards either bacterial or eukaryotic aaRSs. This is a consequence of the use of the same general reaction intermediate (**1**) by aaRSs of all kingdoms of life, despite extensive divergence in the structure and amino acid sequence of these enzymes. These evolved differences have allowed the development of derivatives of aaSA that do show good selectivity for bacteria and that are systemically active in a mouse model of infection.[11,12]

Even though these aaSAs (**2**) are nanomolar inhibitors of aaRS enzymes in vitro, the in vivo antibacterial activity is considerably less, and poor uptake has been suggested as the main reason for this.[2,13,14] Several natural inhibitors of aaRS enzymes circumvent this problem by using a dual active uptake/aaRS inhibition mode of action. Relevant examples of this are the antibiotics agrocin 84 (**5**, Fig. 2) and microcin C (**6**).

Agrocin 84 is a'Trojan Horse' antibiotic with potent activity against *Agrobacterium tumefaciens*, which causes crown gall tumors in a wide variety of plants. The compound resembles a tumor-derived substrate and is actively internalized by *A. tumefaciens*. Once inside the target cell, leucyl-adenylate analogue **7** is liberated and inhibits LeuRS.[15,16] The mode of action of microcin C (**6**) is similar: microcin C is produced by strains of *Escherichia coli* and consists of an N-formylated heptapeptide attached to a modified adenylate moiety. The antibiotic is actively taken up by the Yej transporter, which is present on a number of *Enterobacteriaceae*. Following deformylation by peptide deformylase, the peptide moiety is digested sequentially, starting from the N-terminal methionine. This way, the bacterial peptidases set free aspartyl-adenylate analogue **8**, a potent inhibitor of AspRS.[17–19]

## 2. Results

### 2.1. Design and synthesis

The aim of our research was to find derivatives of 5′-*O*-(*N*-aminoacyl)-sulfamoyladenosine (aaSA, **2**) that show improved antibacterial activity compared to the parent aaSAs. To this end, a series of dipeptidyl-analogues, designed to be taken up by peptide transporters, was prepared.

Ascamycin (**9**, Fig. 3) is a natural antibiotic that closely resembles 5′-*O*-(*N*-L-alanyl)-sulfamoyladenosine (AlaSA), although it has a different mechanism of action. In evaluating several aminoacyl analogues of ascamycin, Ubukata et al. found the L-prolyl-L-prolyl derivative **10** to have a significantly increased whole-cell antibacterial activity against both Gram-positive and Gram-negative bacteria, and they suggested increased uptake by peptide transporters as the reason for this, though this was not further investigated.[20,21]

Our starting hypothesis was that the 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines, which are structurally similar to **10**, would be ta-

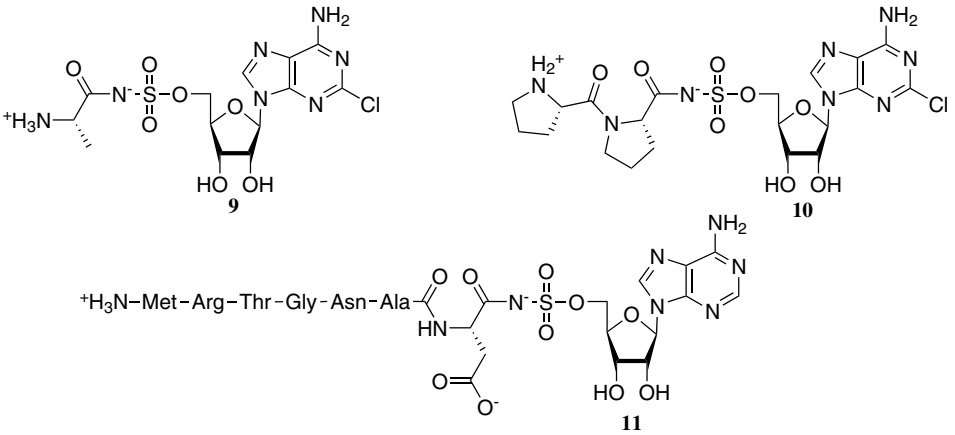
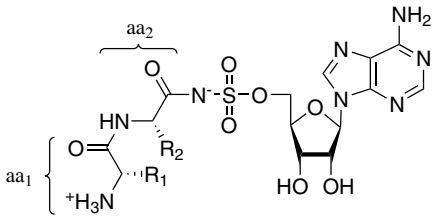



**Figure 3.** Structures of ascamycin (**9**), its L-Pro-L-Pro dipeptidyl derivative **10** and the structure of AspSA derivative **11**, a synthetic analogue of microcin C (**4**).




$R_1$ = side chain of the aminoterminal amino acid ($aa_1$)
$R_2$ = side chain of the carboxyterminal amino acid ($aa_2$)

**Figure 4.** General structure of the 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines **12**–**29**. For the nature of $aa_1$ and $aa_2$: see Table 1.

ken up into microbial cells by dedicated peptide transporters and be digested by intracellular peptidases to yield the active aaSAs, which would in turn inhibit the corresponding aaRS and inhibit bacterial cell growth. Also, these compounds can be regarded as truncated or contracted analogues of synthetic microcin C analogue **11**, which was shown to act via the same mode of action as its natural counterpart **6**. The mode of action of **11** is comparable to that of microcin C, and involves active uptake by the Yej transporter, intracellular digestion by peptidases A, B and N and finally inhibition of AspRS of sensitive *Enterobacteriaceae* [Kazakov et al., in press].

To test this hypothesis, a diverse set of 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines was prepared (Fig. 4 and Table 1). The amino-terminal amino acids were chosen to obtain a set of compounds with sufficient differences in physicochemical parameters such as hydrophobicity, net charge and size. Hereto, the amino acids alanine, methionine, proline, glutamine, lysine and phenylalanine were selected.

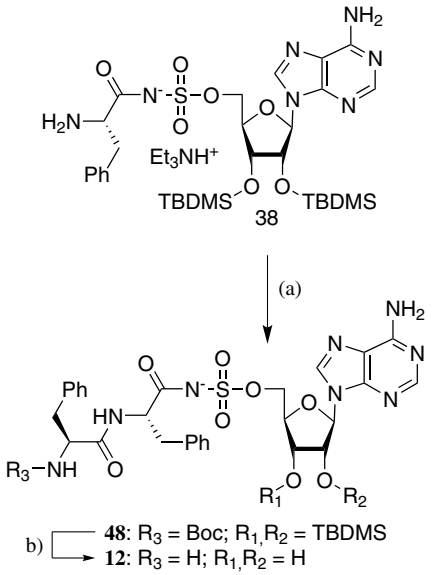
The choice of the carboxy-terminal amino acid was influenced by the results of a preliminary assay (Table S1, Supplementary data), in which all of the 20 analogues of aaSA were tested for their antibacterial activity against a diverse panel of bacteria, both Gram-negative and Gram-positive. In this screening, we found that the aaSA analogues have only limited whole cell antibacterial activity, with no compound showing antibacterial activity below 16 μg/mL. The compounds that showed meaningful activity at 128 μg/mL in this screening were AlaSA, ArgSA, HisSA, LeuSA, LysSA, MetSA and ProSA. From these, the Ala, Leu, Met and Pro analogues were selected. Additionally, we also prepared analogues containing Trp, Phe and Asp as the carboxyterminal amino acid.

Initially, we prepared the 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines by the convergent method developed by Ubukata et al. for their synthesis of **10**.[21] In this synthesis, *N*-Boc-protected dipeptide is activated at the free carboxyl function and reacted with 2′,3′-*O*-isopropylidene-5′-*O*-sulfamoyl-adenosine in the presence of strong base. After removal of the protecting groups using aqueous trifluoroacetic acid, this yields the desired dipeptidyl-sulfamoyladenosine. However, with this method we found the second, carboxyterminal amino acid to be prone to racemization (data not shown). Therefore, we abandoned this method and switched to a serial approach instead. Initially, we coupled *N*-Boc protected amino acid to the respective sugar-protected aaSA using standard peptide coupling procedures, followed by a two step deprotection procedure to remove all protecting groups (Scheme 1). This method was used for the synthesis of PhePheSA (**12**). For the other 5′-*O*-(*N*-dipeptidyl)-sulfamoyladenosines **13**–**29**, this synthesis was further modified, and the *N*-Boc protected amino acid was coupled directly to unprotected aaSA, followed by a short and simple aqueous acid deprotection step to remove the *N*-Boc protecting group

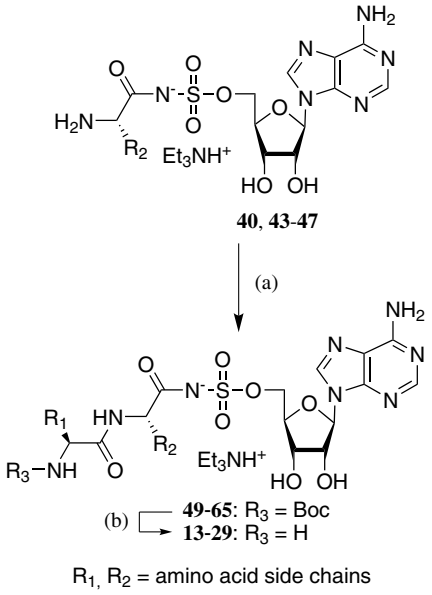
**Table 1**
Overview of the antibacterial activities of dipeptidyl-sulfamoyladenosines **12**–**29** (Fig. 4) and selected reference compounds

| Compd (30 nmol)[c] | $aa_1$[a] | $aa_2$[b] | Growth inhibition area ($mm^2$) | | | |
|---|---|---|---|---|---|---|
| | | | *S. aureus* | *E. faecalis* | *E. coli* wt | *E. coli* ΔABN |
| LeuSA | | Leu | 0 ± 0 | 26.9 ± 5.4[d] | 25.4 ± 5.0[d] | 66 ± 4.2[d] |
| AlaSA | | Ala | 35 ± 3.1[e] | 44.7 ± 11.8[d] | 46.3 ± 6.8[d] | 42.3 ± 3.3[e] |
| MetSA | | Met | 18.7 ± 5.7[e] | 0 ± 0[f] | 26.8 ± 2.6[d] | 40.4 ± 3.3[e] |
| TrpSA | | Trp | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| PheSA | | Phe | 0 ± 0[f] | 40.4 ± 3.3[d] | 38.5 ± 0[d] | 52.7 ± 9.9[d] |
| AspSA | | Asp | 0 ± 0 | 21.3 ± 6.3[e] | 26.8 ± 2.6[d] | 28.3 ± 0[e] |
| **13** | Ala | Leu | 0 ± 0 | 24.2 ± 7.1[d] | 0 ± 0 | |
| **14** | Met | Leu | 0 ± 0 | 33.3 ± 5.1[d] | 0 ± 0 | |
| **15** | Pro | Leu | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **16** | Lys | Leu | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **17** | Glu | Leu | 0 ± 0 | 0 ± 0[f] | 0 ± 0 | |
| **18** | Ala | Ala | 0 ± 0 | 56.9 ± 6.7[d] | 25.3 ± 2.6[d] | 0 ± 0 |
| **19** | Met | Ala | 59 ± 4[e] | 76.1 ± 9.1[e] | 26.8 ± 2.6[d] | 0 ± 0 |
| **20** | Pro | Ala | 0 ± 0 | 0 ± 0[f] | 18.4 ± 2.2[e] | 0 ± 0 |
| **21** | Ala | Met | 0 ± 0 | 0 ± 0[f] | 0 ± 0 | |
| **22** | Met | Met | 0 ± 0 | 83.9 ± 4.6[d] | 0 ± 0 | |
| **23** | Pro | Met | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **24** | Ala | Trp | 0 ± 0 | 0 ± 0 | 0 ± 0 | 0 ± 0[f] |
| **25** | Met | Trp | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **26** | Pro | Trp | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **27** | Lys | Trp | 0 ± 0 | 0 ± 0 | 0 ± 0 | |
| **28** | Glu | Trp | 0 ± 0 | 0 ± 0[f] | 22.4 ± 2.4[d] | 22.4 ± 2.4[e] |
| **12** | Phe | Phe | 0 ± 0 | 26.8 ± 2.6[f] | 18.4 ± 2.2[d] | 19.6 ± 0[e] |
| **29** | Met | Asp | 0 ± 0 | 63.7 ± 7.1[d] | 12.6 ± 0[d] | 31.5 ± 2.8[e] |
| Mupirocin | | NA | >490.93 | 89.4 ± 4.9[d] | 38.5 ± 0[d] | 52.7 ± 9.9[e] |
| MRTGNAD-SA[c] | | Asp | 0 ± 0 | 0 ± 0 | 46.7 ± 12.2[d] | |

Briefly, a lawn of soft Mueller–Hinton agar (0.75% agar) containing bacteria was spread over Mueller–Hinton agar (1.5% agar).
[a] Amino-terminal amino acid.
[b] Carboxyterminal amino acid.
[c] Antibiotic was added as 5 μl drops containing 30 nmol of compound, except for MRTGNAD-SA (1.2 nmol). The plates were incubated at 37 °C for 16–20 h and analyzed. Values are indicated as area in $mm^2$ ± SD. Bacterial strains used were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* and *E. coli* ΔABN.
[d] Clear halos were observed, indicating full growth inhibition.
[e] Opaque halos were observed, indicating partial growth inhibition.
[f] Partial growth inhibition halos were visible but were too faint to be accurately measured.




**Scheme 1.** Synthesis of Phe/Phe analogue (**12**). Reaction conditions: (a) *N*-Boc-L-Phe-OH, HOBt, EDCI·HCl, $Et_3N$, DMF; (b) (i) TFA/$H_2O$ (5:2 v/v), (ii) $Et_3N{\cdot}3HF$, THF.




**Scheme 2.** Synthesis of dipeptidyl-sulfamoyladenosines **13**–**29** of Table 1. Reaction conditions: (a) *N*-Boc-L-amino acid, HOBt, EDCI·HCl, $Et_3N$, DMF; (b) TFA/$H_2O$ (5:2 v/v).

(Scheme 2). We found the latter method to be more time-efficient while keeping the overall yield unchanged.

To our surprise, we were not able to prepare analogues with proline as the carboxyterminal amino acid. We tried to synthesize both the Ala/Pro (**30**) and the Pro/Pro (**31**) analogue via the methods described above, but were not able to isolate the target compounds. The coupling reactions were successful, and both HRMS and NMR showed the presence of the desired compounds. However, extensive decomposition occurred during the subsequent deprotection reaction. In one case, we did observe the desired molecule by mass spectrometric analysis of the partly purified compound but we found the compound to decompose to adenosine during size-exclusion chromatography with $H_2O$/MeOH (3:7 v/v, data not shown). As this suggested instability of aaProSA compounds in water, for which we have no explanation, work on this subset of compounds was abandoned.

### 2.2. Antibacterial evaluation

For the antibacterial evaluation of the compounds, we exposed bacteria in Mueller–Hinton agar to droplets containing 30 nanomoles of the potential antibacterial. After overnight incubation, inhibition zones were determined. All tests were performed with 6 nanomole-doses as well, but here antibacterial activity was very limited (Table S2, supplementary data). Three bacterial species were used for these tests: *Staphylococcus aureus*, *Enterococcus faecialis* and *Escherichia coli*. In addition, a mutant *E. coli* strain lacking broad specificity peptidases A, B and N was subjected to a selection of the compounds, to determine if processing by these peptidases is required for activity of the dipeptidyl-compounds.

The data presented in Table 1 show differing sensitivities amongst the strains tested, with the *S. aureus* strain being clearly less sensitive to the compounds studied. Comparing activity of the aaSA parent compounds, inhibition of the *S. aureus* strain can be detected with 2/6 compounds. In contrast, the *E. faecalis* and *E. coli* strains are both sensitive to 4/6 and 5/6 compounds, respectively. None of the bacterial strains was inhibited by the tryptophanyl analogue.

The situation with the dipeptidyl compounds is more diverse: again, the *S. aureus* strain is resistant to all but one (MetAla analogue **19**), while the *E. faecalis* strain is, to varying extents, inhibited by 7/18 compounds and the *E. coli* wt strain by 6/18.

A number of interesting observations can be made when comparing inhibition between the wt and ΔABN *E. coli* strains: The mutant strain appears to be more sensitive to some of the parent aaSA compounds, and for LeuSA and MetSA this difference is significant ($P = 0.0004$ and 0.005, respectively). The mutant strain has gained resistance towards aa/Ala compounds **18**–**20**, indicating that at least one of the broad specificity peptidases A, B or N is required for digestion of these compounds to the active derivative.[19] In contrast, sensitivity against Glu/Trp analogue **28**, Phe/Phe analogue **12** and Met/Asp analogue **29** is indistinguishable from the wt strain.

Finally, we also tested these strains against the established antibiotic mupirocin and against synthetic McC analogue MRTGNAD-SA (**11**, Fig. 3, [Kazakov2008, in press]). The inhibition zone caused by mupirocin against *S. aureus* was too large to be accurately measured. At the same dose, *E. faecalis* and *E. coli* were also sensitive, albeit a lot less than *S. aureus*. At the low dose tested (1.2 nmol), MRTGNAD-SA was only active against *E. coli*. This is in line with the observation that microcin C is primarily active against *Enterobacteriaceae*.[22]

## 3. Discussion

### 3.1. Variable sensitivity to dipeptidyl analogues

When comparing the inhibition zones between different dipeptidyl compounds and between dipeptidyl compounds and their parent compounds, large differences can be observed: in a number of cases, the addition of a second amino acid to the N-terminal amino acid of aaSA abolished antibacterial activity. However, in other cases, the derivative is much more active than the parent compound: an example from Table 1 is Met/Met analogue **22**: this compound has potent activity against *E. faecalis* but not against any of the other strains tested, while its parent compound MetSA is less active but has a broader spectrum (all three strains are inhibited to some extent). A second example is Met/Asp analogue **29**: compared to the parent compound AspSA, **29** is more active against *E. faecalis* but less active against *E. coli*.

These results point to a potential for modifying the antibacterial spectrum of aaSA compounds by the preparation of dipeptidyl derivatives. This is similar to the results obtained with MRTG-NAD-SA: here, addition of the MRTGNA hexapeptide to AspSA creates a derivative that is much more active against *E. coli*: an inhibition zone of 46.7 ± 12.2 $mm^2$ is produced by only 1.2 nmol of MRTGNAD-SA, compared to an inhibition zone of 26.8 ± 2.6 $mm^2$ produced by 30 nmol of parent compound AspSA. Combined, these results indicate the possibility of generating aminoacyl- and oligopeptidyl-derivatives of compounds with known or expected antibacterial activity to modify the antibacterial efficacy and spectrum of these compounds. It is also important to evaluate the potential immunosuppressive activity of tRNA-synthetase inhibition, as this side effect is unwanted for antibiotics[23].

In addition, aminoacyl- or dipeptidyl-derivation may also be a viable strategy to improve physicochemical characteristics of antibacterial drugs. By coupling with more or less polar amino acids, it is conceivable that oligopeptidyl-derivatives of aaSAs and other antibacterials can be prepared with improved oral availability, water solubility or skin penetration with only limited effects on toxicity.

### 3.2. Influence of peptidases A, B and N

Digestion by peptidases A, B and N is required for activity of some but not all dipeptidyl-sulfamoyl-adenosines: No inhibition zones were found for the aa/Ala compounds **18**–**20**, indicating that digestion by at least one of the missing peptidases is necessary for digestion of these compounds, similar to the situation with MRTG-NAD-SA [Kazakov2008, in press].

In contrast, no difference in sensitivity to Glu/Trp analogue **28**, Phe/Phe analogue **12** and Met/Asp analogue **29** was observed, indicating that these compounds are either digested by enzymes other than peptidases A, B or N, or that these compounds do not require intracellular activation.

A second finding is the increased sensitivity of ΔABN to aaRS inhibitors LeuSA, MetSA, PheSA and mupirocin. For LeuSA ($P = 0.0004$) this difference is significant. A plausible explanation for this increased sensitivity is that the ΔABN *E. coli* strain has a decreased capacity for using the available peptides present in the Mueller–Hinton broth, compared to the wt strain. As a result, the aaRS inhibitors have to compete with lower levels of amino acids and are more efficient in inhibiting bacterial growth.

## 4. Experimental

### 4.1. Chemistry

#### 4.1.1. General information

Reagents and solvents were from commercial suppliers (Acros, Sigma–Aldrich, Bachem, Novabiochem) and used as provided, unless indicated otherwise. DMF and THF were analytical grade and were stored over molecular sieves (4 Å). All other solvents used for reactions were analytical grade and used as provided. Reactions were carried out in oven-dried glassware under a nitrogen atmosphere and stirred at room temperature, unless indicated otherwise.

$^1H$ and $^{13}C$ NMR spectra of the compounds were recorded on a 200 MHz Varian Gemini spectrometer or a Bruker UltraShield Avance 300 MHz spectrometer. Spectra were recorded in DMSO-$d_6$, $D_2O$, or $CDCl_3$. The chemical shifts are expressed as $\delta$ values in parts per million (ppm), using the residual solvent peaks (chloroform: $^1H$, 7.26 ppm, $^{13}C$, 77.36 ppm; DMSO: $^1H$, 2.50 ppm, $^{13}C$, 39.60 ppm; HOD: $^1H$, 4.79 ppm) as a reference. For $^{13}C$ spectra in $D_2O$, ~0.1% dioxane (67.19 ppm) was added as a reference. Coupling constants are given in Hertz (Hz). The peak patterns are indicated by the following abbreviations: br s = broad singlet, d = doublet, m = multiple, q = quadruplet, s = singlet, and t = triplet.

High resolution mass spectra were recorded on a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard ESI interface; samples were infused in 2-propanol/$H_2O$ (1:1, v/v) at 3 μL $min^{-1}$.

For TLC, precoated aluminum sheets were used (Merck, Silica Gel 60 $F_{254}$). The spots were visualized by UV light. Column chromatography was performed on ICN Silica Gel 60A 60–200. For size exclusion chromatography, a 2 × 30 cm column of Sephadex LH-20 was used as the solid phase and MeOH/$H_2O$ (7: 3, v/v) as the eluent. Eluent compositions are expressed as v/v.

Several of the final compounds were found to contain low but varying amounts of the counterions triethylammonium and *N*-(chloromethyl)-*N*,*N*-diethylethanaminium. To compensate for this in the biological assays, concentrations of compounds were determined spectrophotometrically (UV, $\lambda = 260$ nm) and purified *N*-(chloromethyl)-*N*,*N*-diethylethanaminium chloride salt was subjected to the biological assays as well.

#### 4.1.2. Preparation of 5′-*O*-(*N*-L-aminoacyl)-sulfamoyladenosines

**4.1.2.1. 2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[*N*-(*N*-Boc-L-tryptophanyl)-sulfamoyl]adenosine ($Et_3N$ salt, 33).** To an ice-cooled solution of 2′,3′-di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-sulfamoyladenosine (**32**, 4.60 g, 8.00 mmol, 1.0 equiv) and *N*-Boc-L-tryptophan *N*-hydroxysuccinimide ester (3.53 g, 8.79 mmol, 1.1 equiv) in DMF (30 mL) was added DBU (2.0 g, 13.13 mmol, 1.6 equiv) and the reaction mixture was allowed to come to rt overnight. Next, the volatiles were removed in vacuo and the residue was purified by silica gel chromatography ($Et_3N$ 1%, MeOH 2.5→10% in $CH_2Cl_2$) to yield 7.50 g (7.79 mmol, 97%) of **33**.

$^1H$ NMR (DMSO-$d_6$): $\delta$ −0.41 (s, 3H, Si—$CH_3$), −0.07 (s, 3H, Si—$CH_3$), 0.12 (s, 3H, Si—$CH_3$), 0.14 (s, 3H, Si—$CH_3$), 0.67 (s, 9H, Si—C—$(CH_3)_3$), 0.92 (s, 9H, Si—C—$(CH_3)_3$), 1.15 (t, 9H, $Et_3NH^+$—$H_3$, $J = 7.3$ Hz), 1.32 (s, 9H, Boc—C—$(CH_3)_3$), 2.94–3.15 (m, 7H, $Et_3NH^+$—$H_2$, Trp-β-$H_A$) 3.26 (dd, 1H, Trp-β-$H_B$, $J = 4.2$ Hz, $J = 14.3$ Hz), 3.97–4.27 (m, 4H, 4′-H, 5′-$H_2$, Trp-α-H), 4.36 (d, 1H, 3′-H, $J = 4.4$ Hz), 4.92 (dd, 1H, 2′-H, $J = 4.4$ Hz, $J = 7.3$ Hz), 5.86 (d, 1H, Trp-amide-NH, $J = 7.4$ Hz), 5.97 (d, 1H, 1′-H, $J = 7.3$ Hz), 6.87–7.12 (m, 3H, Trp-2H, Trp-5H, Trp-6H), 7.26 (s, 2H, Ade-$NH_2$), 7.30 (d, 1H, Trp-7-H, $J = 8.0$ Hz), 7.49 (d, 1H, Trp-4-H, $J = 7.8$ Hz), 8.14 (s, 1H, 2-H), 8.52 (s, 1H, 8-H), 10.7 (s, 1H, Trp-NH).

$^{13}C$ NMR (DMSO-$d_6$): $\delta$ −5.7, 8.7, 17.8, 25.8, 45.8, 66.8, 74.8, 84.1, 110.8, 118.0, 118.9, 123.4, 135.9, 149.9, 154.7, 176.0.

HRMS for $C_{38}H_{59}N_8O_9SSi_2$ $[M-H]^-$ calcd: 859.3664; found: 859.3668.

**4.1.2.2. 5′-*O*-(*N*-L-Tryptophanyl)-sulfamoyladenosine ($Et_3N$ salt, 34a and Na salt, 34b).** Compound **33** (7.40 g, 7.69 mmol, 1.0 equiv) in TFA/$H_2O$ (30 mL, 5:2, v/v) was stirred for 30 min at rt. Next, the volatiles were evaporated, coevaporated twice with EtOH and once with EtOH + $Et_3N$ (10 mL), to neutralize any remaining acid. The residue was dried for 48 h ($P_2O_5$, in vacuo, rt) and $Et_3N \cdot 3HF$ (2.5 mL, 15.3 mmol, 6.0 equiv) was added, followed by THF (150 mL, added in portions) and the reaction mixture was stirred overnight. As TLC analysis (HOAc 1%, MeOH 20% in $CH_2Cl_2$) showed remaining partially protected compound, more $Et_3N \cdot 3HF$ (2 mL, 12.2 mmol, 4.8 equiv) was added and the reaction was continued for an additional 4 h. Next, $Et_3N$ (10 mL) was added and the volatiles were removed in vacuo. The residual oil was partly purified on a short silica gel column ($Et_3N$ 1%, MeOH 5→30% in $CH_2Cl_2$). Fractions containing the title compound were collected and evaporated, and subjected to silica gel chromatography ($Et_3N$ 1%, MeOH 10→30% in $CH_2Cl_2$) to afford 4.46 g (7.04 mmol, 92%) of **34a**. The compound was used as such for fur-

ther syntheses. For analytical purposes and for biological assays, a small portion of **34a** was precipitated as the sodium salt **34b** by adding acetone to a solution of **34a** and NaOH (1.2 equiv) in water.

$^1$H NMR (**40b**, DMSO-$d_6$ + ($D_2O$)): $\delta$ 3.05 (dd, 1H, β-$H_A$, $J$ = 8.6 Hz, $J$ = 15.1 Hz), 3.68 (dd, 1H, α-H, $J$ = 4.5 Hz, $J$ = 8.2 Hz), 4.03–4.24 (m, 5H, 3′-H, 4′-H, 5′-$H_2$), 4.61 (t, 1H, 2′-H, $J$ = 5.3 Hz), 5.92 (d, 1H, 1′-H, $J$ = 5.8 Hz), 6.99 (m, 1H, Trp-5H), 7.05 (m, 1H, Trp-6H), 7.23 (d, 1H, Trp-2H, $J$ = 12.6 Hz), 7.35 (d, 1H, Trp-7H, $J$ = 7.9 Hz), 7.57 (d, 1H, Trp-4-H, $J$ = 7.8 Hz), 8.14 (s, 1H, 2-H), 8.40 (s, 1H, 8-H).

$^{13}$C NMR (**40b**, DMSO-$d_6$): $\delta$ 27.4, 55.7, 67.6, 70.8, 73.6, 82.6, 87.2, 108.2, 111.5, 118.4, 118.6, 119.0, 121.1, 124.6, 127.3, 136.4, 139.5, 149.7, 152.8, 156.1, 172.5.

HRMS for $C_{21}H_{25}N_8O_7S$ [M+H]$^+$ calcd: 533.1569; found: 533.1569.

**4.1.2.3. 2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[*N*-(*N*-Boc-L-phenylalanyl)-sulfamoyl]adenosine (Et$_3$N salt, 35).** Following the procedure used for the synthesis of **33**, 2′,3′-di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-sulfamoyladenosine (**32**, 575 mg, 1.00 mmol, 1.0 equiv) and *N*-Boc-L-phenylalanine *N*-hydroxysuccinimide ester (435 mg, 1.20 mmol, 1.2 equiv) were reacted to give 892 mg (0.96 mmol, 97%) of **35**.

$^1$H NMR (DMSO-$d_6$): $\delta$ −0.42 (s, 3H, Si—$CH_3$), −0.08 (s, 3H, Si—$CH_3$), 0.11 (s, 3H, Si—$CH_3$), 0.13 (s, 3H, Si—$CH_3$), 0.66 (s, 9H, Si—C—$(CH_3)_3$), 0.92 (s, 9H, Si—C—$(CH_3)_3$), 1.15 (t, 9H, $Et_3NH^+$—$H_3$, $J$ = 7.3 Hz), 1.31 (s, 9H, Boc—C—$(CH_3)_3$), 2.67–3.15 (m, 8H, $Et_3NH^+$—$H_3$, β-$H_2$), 3.87–4.24 (m, 4H, 4′-H, 5′-$H_2$, Phe-α-H), 4.34 (d, 1H, 3′-H, $J$ = 4.4 Hz), 4.92 (dd, 1H, 2′-H, $J$ = 4.4 Hz, $J$ = 7.3 Hz), 5.96 (d, 1H, 1′-H, $J$ = 7.2 Hz), 7.13–7.36 (m, 7H, phenyl-H, Ade-$NH_2$), 8.13 (s, 1H, 2-H), 8.49 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ −5.7, −4.7, 9.3, 17.4, 17.8, 25.5, 25.8, 28.2, 36.8, 45.7, 57.5, 66.9, 73.4, 74.7, 77.53, 84.2, 86.1, 119.0, 126.0, 128.0, 128.2, 129.4, 129.6, 139.0 and 139.7, 150.1, 152.9, 154.9, 156.2, 175.6.

HRMS for $C_{36}H_{60}N_7O_9SSi_2$ [M+H]$^+$ calcd: 822.3712; found: 822.3717.

**4.1.2.4. 2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[*N*-(L-phenylalanyl)-sulfamoyl]adenosine (Et$_3$N salt, 36).** Compound **35** was dissolved in TFA/$H_2O$ (5:2 v/v, 5 mL) and stirred for 2 h at rt. Next, the volatiles were evaporated, coevaporated twice with EtOH and once with EtOH + $Et_3N$ (10 mL), to neutralize any remaining acid. The residue was purified by silica gel chromatography ($Et_3N$ 1%, MeOH 5→10% in $CH_2Cl_2$) to give 389 mg of **36**.

$^1$H NMR (DMSO-$d_6$): $\delta$ −0.38 (s, 3H, Si—$CH_3$), −0.09 (s, 3H, Si—$CH_3$), 0.11 (s, 3H, Si—$CH_3$), 0.13 (s, 3H, Si—$CH_3$), 0.69 (s, 9H, Si—C—$(CH_3)_3$), 0.92 (s, 9H, Si—C—$(CH_3)_3$), 1.18 (t, $Et_3NH^+$—$H_3$, $J$ = 7.3 Hz), 2.85–3.30 (m, $Et_3NH^+$—$H_3$, β-$H_2$), 3.69 (m, 1H, α-H), 3.94-4.55 (m, 4H, 4′-H, 5′-$H_2$, 3′-H), 4.92 (dd, 1H, 2′-H, $J$ = 4.4 Hz, $J$ = 7.0 Hz), 5.96 (d, 1H, 1′-H, $J$ = 6.8 Hz), 7.14-7.46 (m, 7H, phenyl-H, Ade-$NH_2$), 8.17 (s, 1H, 2-H), 8.49 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ −5.6, −5.4, −4.8, 8.5, 17.4, 17.7, 25.5, 25.7, 37.1, 45.7, 56.2, 67.3, 73.1, 74.6, 83.7, 86.6, 119.1, 126.9, 128.5, 129.7, 36.3, 139.9, 149.8, 152.5, 156.0, 172.0.

HRMS for $C_{31}H_{52}N_7O_7SSi_2$ [M+H]$^+$ calcd: 722.3187; found: 722.3166.

**4.1.2.5. 5′-*O*-[*N*-(L-Phenylalanyl)-sulfamoyl]adenosine (Et$_3$N salt, 37).** Compound **35** (500 mg, 0.61 mmol, 1.0 equiv) was dissolved in TFA/$H_2O$ (5:2, v/v, 5 mL) and stirred for 1.5 h at rt. Next, the volatiles were evaporated, coevaporated twice with EtOH and once with EtOH + $Et_3N$ (10 mL). After removal of the volatiles, the residue was dissolved in THF (20 mL) and $Et_3N \cdot 3HF$ (400 μL, 2.45 mmol, 12 equiv) was added and the mixture was stirred overnight at rt. After evaporation of the volatiles, the residue was subjected to silica gel chromatography (MeOH 20% in $CHCl_3$, v/v). The residue obtained this way was further purified by size exclusion chromatography to give 133 mg (0.27 mmol, 44%) of **37**.

$^1$H NMR (DMSO-$d_6$): $\delta$ 2.93 (dd, 1H, β-$H_A$, $J$ = 7.8, 14.2 Hz), 3.14 (dd, 1H, β-$H_B$, $J$ = 4.9, 14.2 Hz), 3.66 (dd, 1H, α-H, $J$ = 5.0, 7.7 Hz), 3.95–4.21 (m, 4H, 3′-H, 4′-H, 5′-$H_2$), 4.62 (t, 1H, 2′-H, $J$ = 5.3 Hz), 5.92 (d, 1H, 1′-H, $J$ = 5.8 Hz), 7.15-7.35 (m, 7H, phenyl-H, Ade-$NH_2$), 8.14 (s, 1H, 2-H), 8.46 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 37.4, 56.3, 67.6, 70.8, 73.5, 82.5, 87.2, 119.0, 126.8, 128.5, 129.6, 136.3, 139.5, 149.6, 152.7, 156.1, 172.2.

HRMS for $C_{19}H_{24}N_7O_7S$ [M+H]$^+$ calcd: 494.1458; found: 494.14491.

The compounds 5′-*O*-(*N*-L-alanyl)-sulfamoyladenosine (**38**), 5′-*O*-(*N*-L-leucyl)-sulfamoyladenosine (**39**), 5′-*O*-(*N*-L-methionyl)-sulfamoyladenosine (**40**), 5′-*O*-(*N*-L-aspartyl)-adenosine (**41**) were synthesized by the methods described above for the synthesis of TrpSA and PheSA. Analytical data of the final compounds were in agreement with literature values.[6,10,23–27]

### 4.1.3. Preparation of 5′-O-(*N*-dipeptidyl)-sulfamoyladenosines 12–29

**4.1.3.1. 2′,3′-Di-*O*-(*tert*-butyldimethylsilyl)-5′-*O*-[*N*-[*N*-(*N*-Boc-L-phenylalanyl)-L-phenylalanyl]-sulfamoyl]adenosine (Et$_3$N salt, 42).** A solution of **36** (375 mg, 0.46 mmol, 1.0 equiv), *N*-Boc-L-phenylalanine (179 mg, 0.68 mmol, 1.5 equiv) and HOBt (50 mg, 0.37 mmol, 0.8 equiv) in DMF (5 mL) was cooled to 0 °C and EDCI·HCl (104 mg, 0.54 mmol, 1.2 equiv) was added. The mixture was stirred and allowed to come to rt overnight. Next, the volatiles were removed in vacuo and the residue was purified by silica gel column chromatography ($Et_3N$ 1%, MeOH 5% in $CH_2Cl_2$), yielding 470 mg (0.44 mmol, 96%) of **42**.

HRMS for $C_{45}H_{69}N_8O_{10}SSi_2$ [M+H]$^+$ calcd: 969.4396; found: 969.4408.

**4.1.3.2. 5′-*O*-[*N*-(*N* -L-Phenylalanyl)-L-phenylalanyl]-sulfamoyl-adenosine (12).** Compound **42** (470 mg, 0.44 mmol, 1.0 equiv) was treated with TFA/$H_2O$ (5:2, v/v, 5 mL) for 2 h at rt. Next, the volatiles were evaporated, coevaporated twice with EtOH and once with EtOH + $Et_3N$ (1 mL). After drying (16 h, in vacuo, $P_2O_5$), the residue was dissolved in THF (20 mL) and $Et_3N \cdot 3HF$ (381 μL, 2.34 mmol, 16.0 equiv) was added. The reaction mixture was stirred overnight at rt. Next, $Et_3N$ (0.5 mL) was added and the volatiles were removed in vacuo. The residue was purified by silica gel chromatography ($Et_3N$ 1%, MeOH 5→10% in $CH_2Cl_2$) to give 162 mg (0.21 mmol, 47%) of **12**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 2.70–2.91 (m, 2H, 2 × Phe-β-H), 3.00–3.22 (m, 2H, 2 × Phe-β-H), (m, 1H, Phe1-α-H), 3.94–4.18 (m, 4H, 4′-H, 5′-$H_2$, Phe2-α-H), 4.28 (dd, 1H, 3′-H, $J$ = 4.7, 8.1 Hz), 4.59 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.91 (d, 1H, 1′-H, $J$ = 6.1 Hz), 7.10–7.35 (m, 10H, Aryl-H), 8.13 (s, 1H, 2-H), 8.39 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 37.9, 38.3, 53.9, 56.9, 67.4, 71.0, 73.6, 82.8, 86.9, 118.9, 126.0, 126.9, 128.0, 128.5, 129.4, 129.7, 138.7, 139.4, 149.7, 152.7, 156.1, 174.8.

HRMS for $C_{28}H_{31}N_8O_8S$ [M−H]$^-$ calcd: 639.1986; found: 639.1989.

**4.1.3.3. 5′-*O*-[*N*-[*N*-(*N* -Boc-L-alanyl)-L-leucyl]-sulfamoyl]adenosine (Et$_3$N salt, 43).** A solution of **39** (230 mg, 0.41 mmol, 1.0 equiv), *N*-Boc-L-alanine (85 mg, 0.45 mmol, 1.1 equiv) and HOBt (50 mg, 0.37 mmol, 0.9 equiv) in DMF (5 mL) was cooled to 0 °C and EDCI·HCl (86 mg, 0.45 mmol, 1.1 equiv) was added. The mixture was allowed to come to rt and stirred overnight. Next, the volatiles were removed in vacuo and the residue was purified by silica gel column chromatography ($Et_3N$ 1%, MeOH 10→20% in $CH_2Cl_2$), yielding 293 g (0.40 mmol, 98%) of **43**.

HRMS for $C_{24}H_{37}N_8O_{10}S$ $[M-H]^-$ calcd: 629.2354; found: 629.2352.

**4.1.3.4. 5′-*O*-[*N*-(*N*-L-Alanyl)-L-leucyl]-sulfamoyladenosine (13).** Compound **43** (280 mg, 0.38 mmol, 1.0 equiv) was dissolved in ice-cold TFA/$H_2O$ 5:2 (5 mL, 5:2, v/v) and the resulting solution was stirred and allowed to come to rt. After 1.5 h, the volatiles were removed and the residue was coevaporated twice with EtOH and once with $Et_3N$ (1 mL). The volatiles were removed again and the residue was purified by silica gel chromatography ($Et_3N$ 1%, MeOH 10→20% in $CH_2Cl_2$) and further purified by size exclusion chromatography to yield 120 mg (0.23 mmol, 61%) of **13**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 0.83 (m, 6H, Leu2-$\delta-H_3$), 1.28–1.45 (m, 4H, Ala1-$H_3$, Leu2-β-$H_A$), 1.45–1.66 (m, 2H, Leu2-β-$H_B$, Leu2-γ-H), 3.79 (q, 1H, Ala1-α-H, $J$ = 7.0 Hz), 3.93–4.18 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Leu2-α-H), 4.57 (t, 1H, 2′-H, $J$ = 5.4 Hz), 5.90 (d, 1H, 1′-H, $J$ = 5.9 Hz), 8.14 (s, 1H, 2-H), 8.37 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 17.6, 21.9, 23.3, 24.4, 42.2, 48.3, 54.0, 67.3, 71.0, 73.6, 82.8, 87.0, 119.0, 139.5, 149.7, 152.7, 156.1, 169.0, 176.2.

HRMS for $C_{19}H_{31}N_8O_8S$ $[M+H]^+$ calcd: 531.1985; found: 531.1979.

**4.1.3.5. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-methionyl)-L-leucyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 44).** Following the procedure used for the synthesis of **43**, **39** (250 mg, 0.45 mmol, 1.0 equiv) and *N*-Boc-L-methionine (125 mg, 0.50 mmol, 1.1 equiv) were reacted to yield 330 mg (0.42 mmol, 93%) of **44**.

HRMS for $C_{26}H_{43}N_8O_{10}S_2$ $[M+H]^+$ calcd: 691.2543; found: 691.2548.

**4.1.3.6. 5′-*O*-[*N*-(*N*-L-Methionyl)-L-leucyl]-sulfamoyladenosine (14).** Following the procedure used for the synthesis of **13**, **44** (325 mg, 0.41 mmol, 1.0 equiv) was deprotected to yield 162 mg (0.27 mmol, 67%) of **14**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 0.83 (m, 6H, Leu2-$\delta-H_3$), 1.30–1.46 (m, 1H, Leu2-β-$H_A$), 1.46–1.67 (m, 2H, Leu2-β-$H_B$, Leu2-γ-H), 1.76–2.08 (m, 5H, Met1-*S*-$H_3$, Met1-β-$H_2$), 2.52 (partly obscured by DMSO-$d_6$-peak, Met1-γ-$H_2$), 3.73 (m, 1H, Met1-α-H, visible before addition of $D_2O$), 3.93–4.18 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Leu2-α-H), 4.56 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.90 (d, 1H, 1′-H, $J$ = 6.0 Hz), 8.14 (s, 1H, 2-H), 8.37 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 14.5, 21.9 and 23.3, 24.4, 28.5, 31.9, 42.1, 52.1, 53.8, 67.3, 71.0, 73.6, 82.8, 86.9, 119.0, 139.5, 149.7, 152.7, 156.1, 168.5, 176.1.

HRMS for $C_{21}H_{33}N_8O_8S_2$ $[M-H]^-$ calcd: 589.1863; found: 589.1840.

**4.1.3.7. 5′-*O*-[*N*-[*N*-(*N* -Boc-L-Prolyl)-L-leucyl]-sulfamoyl]adenosine ($Et_3N$ salt, 45).** Following the procedure used for the synthesis of **43**, **39** (280 mg, 0.50 mmol, 1.0 equiv) and *N*-Boc-L-proline (85 mg, 0.55 mmol, 1.1 equiv) were reacted to yield 381 mg (0.50 mmol, 100%) of **45**.

HRMS for $C_{26}H_{39}N_8O_{10}S$ $[M-H]^-$ calcd: 655.2510; found: 655.2488.

**4.1.3.8. 5′-*O*-[*N*-(*N*-L-Prolyl)-L-leucyl]-sulfamoyladenosine (15).** Following the procedure used for the synthesis of **13**, **45** (375 mg, 0.49 mmol, 1.0 equiv) was deprotected to yield 184 mg (0.33 mmol, 67%) of **15**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 0.85 (m, 6H, Leu2-$\delta-H_3$), 1.32–1.48 (m, 1H, Leu2-β-$H_A$), 1.52–1.67 (m, 2H, Leu2-β-$H_B$, Leu2-γ-H), 1.73–1.87 (m, 2H, Pro1-$\gamma-H_2$), 1.87–2.01 (m, 1H, Pro1-β-$H_A$), 2.15–2.32 (m, 1H, Pro1-β-$H_B$), 3.2 (m, partly obscured by residual $Et_3NH^+-H_2$, Pro1-$\delta-H_2$), 3.93-4.19 (m, 6H, 3′-H, 4′-H, 5′-$H_2$, Leu2-α-H, Pro1-α-H), 4.60 (t, 1H, 2′-H, $J$ = 5.3 Hz), 5.91 (d, 1H, 1′-H, $J$ = 6.0 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 21.9, 23.3, 23.7, 24.5, 29.7, 42.0, 45.9, 54.0, 59.1, 67.3, 71.0, 73.6, 82.8, 87.0, 119.0, 139.5, 149.7, 152.7, 156.1, 167.9, 176.1.

HRMS for $C_{21}H_{32}N_8O_8S$ $[M+H]^+$ calcd: 557.2142; found: 557.2145.

**4.1.3.9. 5′-*O*-[*N*-[*N*-($N_\alpha$,$N_\epsilon$-Di-Boc-L-lysyl)-L-leucyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 46).** Following the procedure used for the synthesis of **43**, **39** (260 mg, 0.46 mmol, 1.0 equiv) and $N_\alpha$,$N_\epsilon$-di-Boc-L-lysine (173 mg, 0.50 mmol, 1.1 equiv) were reacted to yield 310 mg (0.35 mmol, 76%) of **46**.

HRMS for $C_{32}H_{52}N_9O_{12}S$ $[M-H]^-$ calcd: 786.3456; found: 786.3429.

**4.1.3.10. 5′-*O*-[*N*-(*N*-L-Lysyl)-L-leucyl]-sulfamoyladenosine (16).** Following the procedure used for the synthesis of **13**, **46** (305 mg, 0.34 mmol, 1.0 equiv) was deprotected. For silica gel chromatography $Et_3N$ 1%, MeOH 10→70% in $CH_2Cl_2$ was used. This way, 66 mg (0.11 mmol, 32%) of **16** was obtained.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 0.85 (m, 6H, Leu2-$\delta-H_3$), 1.13–1.68 (m, 9H, Lys1-β-$H_2$, Lys1-γ-$H_2$, Lys1-δ-$H_2$, Leu2-β-$H_2$, Leu2-γ-H), 2.75 (t, 2H, Lys1-–$H_2$, $J$ = 6.5 Hz), 3.25 (t, 1H, Lys1-α-H, $J$ = 5.8 Hz), 3.96–4.20 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Leu2-α-H), 4.58 (t, 1H, 2′-H, $J$ = 5.2 Hz), 5.91 (d, 1H, 1′-H, $J$ = 5.6 Hz), 8.15 (s, 1H, 2-H), 8.43 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 21.6, 22.0, 23.3, 24.5, 26.9, 33.9, 42.4, 48.7, 53.4, 53.8, 67.3, 70.8, 73.7, 82.5, 87.1, 119.0, 139.5, 149.6, 152.7, 156.1, 173.3, 177.4.

HRMS for $C_{22}H_{38}N_9O_8S$ $[M+H]^+$ calcd: 588.2564; found: 588.2564.

**4.1.3.11. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-Glutamyl-γ-*O*-*tert*-butyl ester)-L-leucyl]-sulfamoyl]adenosine ($Et_3N$ salt; 47).** Following the procedure used for the synthesis of **43**, **39** (250 mg, 0.45 mmol, 1.0 equiv) and *N*-Boc-L-glutamic acid γ-*O*-*tert*-butyl ester (152 mg, 0.50 mmol, 1.1 equiv) were reacted to yield 347 mg (0.41 mmol, 91%) of **47**.

HRMS for $C_{27}H_{41}N_8O_{12}S$ $[M-H]^-$ calcd: 701.2565; found: 701.2560.

**4.1.3.12. 5′-*O*-[*N*-(*N*-L-Glutamyl)-L-leucyl]-sulfamoyladenosine ($Et_3N$ salt, 17).** Following the procedure used for the synthesis of **13**, **47** (342 mg, 0.40 mmol, 1.0 equiv) was deprotected. For silica gel chromatography $Et_3N$ 1%, MeOH 10→50% in $CH_2Cl_2$ was used. This way, 143 mg (0.21 mmol, 52%) of **17** was obtained.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 0.86 (m, 6H, Leu2-$\delta-H_3$), 1.15 (t, $Et_3NH^+-H_3$, $J$ = 7.3 Hz), 1.19–1.70 (m, 3H, Leu2-β-$H_2$, Leu2-γ-H), 1.89 (m, 2H, Glu1-β-$H_2$), 2.40 (t, 2H, Glu1-γ-$H_2$, $J$ = 7.6 Hz), 2.96 (q, $Et_3NH^+-H_2$, $J$ = 7.3 Hz), 3.69 ( before addition of $D_2O$: t, 1H, Glu1-α-H, $J$ = 6.1 Hz), 3.92–4.18 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Leu2-α-H), 4.58 (t, 1H, 2′-H, $J$ = 5.1 Hz), 5.90 (d, 1H, 1′-H, $J$ = 6.1 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 9.0, 21.8, 23.3, 24.4, 27.6, 30.7, 42.1, 45.5, 52.3, 53.8, 67.3, 71.0, 73.7, 82.8, 86.9, 118.9, 139.4, 149.7, 152.7, 156.1, 168.8, 174.5, 176.2.

HRMS for $C_{21}H_{32}N_8O_{10}S$ $[M-H]^-$ calcd: 587.1884; found: 587.1863.

**4.1.3.13. 5′-*O*-[*N*-[*N*-(*N* -Boc-L-Alanyl)-L-alanyl]-sulfamoyl]adenosine ($Et_3N$ salt, 48).** Following the procedure used for the synthesis of **43**, **38** (240 mg, 0.46 mmol, 1.0 equiv) and *N*-Boc-L-alanine (87 mg, 0.46 mmol, 1.0 equiv) were reacted to yield 211 mg (0.31 mmol, 67%) of **48**.

ESI-MS for $C_{21}H_{33}N_8O_{10}S$ $[M+H]^+$ calcd: 589.2; found: 589.1.

**4.1.3.14. 5′-*O*-[*N*-(*N*-L-Alanyl)-L-alanyl]-sulfamoyladenosine ($Et_3N$ salt, 18).** Following the procedure used for the synthesis of **13**, **48** (205 mg, 0.30 mmol, 1.0 equiv) was deprotected to yield 118 mg (0.20 mmol, 67%) of **18**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.17 (t, $Et_3NH^+$—$H_3$, $J$ = 7.2 Hz), 1.23 (d, 3H, Ala2-β-$H_3$, $J$ = 6.6 Hz), 1.33 (d, 1H, Ala1-β-$H_3$, $J$ = 6.5 Hz), 3.05 (q, 6H, $Et_3NH^+$—$H_2$, $J$ = 7.2 Hz), 3.77–3.85 (t, 1H, Ala1-α-H, $J$ = 6.7 Hz), 3.95–4.21(m, 5H, 3′-H, 4′-H, 5′-$H_2$, Ala2-α-H), 4.57 (t, 1H, 2′-H, $J$ = 5.0 Hz), 5.91 (d, 1H, 1′-H, $J$ = 5.8 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 8.7, 17.4, 19.3, 45.5, 48.2, 51.0, 67.3, 71.0, 73.6, 82.8, 87.0, 119.0, 139.5, 149.7, 152.7, 156.1, 168.5, 176.0.

HRMS for $C_{16}H_{24}N_8O_8S$ [M−H]$^-$ calcd: 487.1360; found: 487.1357.

**4.1.3.15. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-methionyl)-L-alanyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 49).** Following the procedure used for the synthesis of **43**, **38** (270 mg, 0.52 mmol, 1.0 equiv) and *N*-Boc-L-methionine (130 mg, 0.52 mmol, 1.0 equiv) were reacted to yield 269 mg (0.36 mmol, 69%) of **49**.

HRMS for $C_{23}H_{35}N_8O_{10}S_2$ [M−H]$^-$ calcd: 647.1918; found: 647.1920.

**4.1.3.16. 5′-*O*-[*N*-(*N*-L-Methionyl)-L-alanyl]-sulfamoyladenosine ($Et_3N$ salt, 19).** Following the procedure used for the synthesis of **13**, **49** (264 mg, 0.35 mmol, 1.0 equiv) was deprotected to yield 177 mg (0.27 mmol, 78%) of **19**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.10–1.34 (m, $Et_3NH^+$—$H_3$, Ala2-β-$H_3$), 1.88–2.17 (m, 5H, Met1-*S*-$H_3$, Met1-β-$H_2$), 2.56 (partly obscured by DMSO-$d_6$-peak, Met1-$\gamma-H_2$), 3.05 (q, 2 × $Et_3NH^+$—$H_2$, $J$ = 7.2 Hz), 3.83 (t, 1H, Met1-α-H, $J$ = 5.9 Hz), 3.94–4.21 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Ala2-α-H), 4.58 (m, 1H, 2′-H), 5.91 (d, 1H, 1′-H, $J$ = 5.8 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 8.6, 14.5, 19.1, 28.3, 31.3, 45.6, 51.0, 51.6, 67.4, 70.9, 73.6, 82.8, 87.0, 119.0, 139.5, 149.7, 152.8, 156.0, 167.2, 176.0.

HRMS for $C_{18}H_{28}N_8O_8S_2$ [M−H]$^-$ calcd: 547.1394; found: 547.1379.

**4.1.3.17. 5′-*O*-[*N*-[*N*-(*N* -Boc-L-prolyl)-L-alanyl]-sulfamoyl]adenosine ($Et_3N$ salt, 50).** Following the procedure used for the synthesis of **43**, **38** (213 mg, 0.41 mmol, 1.0 equiv) and *N*-Boc-L-proline (88 mg, 0.41 mmol, 1.0 equiv) were reacted to yield 254 mg (0.35 mmol, 87%) of **50**.

HRMS for $C_{23}H_{33}N_8O_{10}S$ [M−H]$^-$ calcd: 613.2041; found: 613.2035.

**4.1.3.18. 5′-*O*-[*N*-(*N*-L-Prolyl)-L-alanyl]-sulfamoyladenosine ($Et_3N$ salt, 20).** Following the procedure used for the synthesis of **13**, **50** (250 mg, 0.35 mmol, 1.0 equiv) was deprotected to yield 150 mg (0.24 mmol, 69%) of **20**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.16 (t, $Et_3NH^+$—$H_3$, $J$ = 7.3 Hz), 1.22 (d, Ala2-β-$H_3$, $J$ = 4.7 Hz), 1.75–2.06 (m, 3H, Pro1-β-$H_A$, Pro1-$\gamma-H_2$), 2.18-2.33 (m, 1H, Pro1-β-$H_A$), 2.94–3.27 (m, $Et_3NH^+$—$H_2$, Pro1-$\delta-H_2$), 3.92–4.22 (m, 7H, 3′-H, 4′-H, 5′-$H_2$, Ala2-α-H, Pro1-α-H), 4.61 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.90 (d, 1H, 1′-H, $J$ = 6.1 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 8.6, 19.1, 23.6, 29.6, 45.6, 45.9, 51.2, 59.0, 67.4, 71.0, 73.6, 82.8, 87.0, 119.0, 139.5, 149.7, 152.7, 156.1, 167.3, 175.9.

HRMS for $C_{18}H_{26}N_8O_8S$ [M−H]$^-$ calcd: 513.1516; found: 513.1512.

**4.1.3.19. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-alanyl)-L-methionyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 51).** Following the procedure used for the synthesis of **43**, **40** (266 mg, 0.46 mmol, 1.0 equiv) and *N*-Boc-L-alanine (116 mg, 0.61 mmol, 1.3 equiv) were reacted to yield 303 mg (0.40 mmol, 87%) of **51**.

HRMS for $C_{23}H_{35}N_8O_{10}S_2$ [M−H]$^-$ calcd: 647.1996; found: 647.1912.

**4.1.3.20. 5′-*O*-[*N*-(*N*-L-Alanyl)-L-methionyl]-sulfamoyladenosine ($Et_3N$ salt, 21).** Following the procedure used for the synthesis of **13**, **51** (298 mg, 0.40 mmol, 1.0 equiv) was deprotected to yield 63 mg (0.10 mmol, 24%) of **21**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.14 (t, $Et_3NH^+$—$H_3$, $J$ = 7.1 Hz), 1.33 (d, 3H, Ala1-β-$H_3$, $J$ = 6.6 Hz), 1.67–1.88 (m, 1H, Met2-β-$H_A$), 1.88–2.06 (m, 4H, Met2-β-$H_A$, Met-*S*-$CH_3$), 2.43 (m, 2H, Met2-γ-$H_2$) 3.03 (q, $Et_3NH^+$—$H_2$, $J$ = 7.1), 3.78–3.92 (m, 1H, Ala1-α-H), 3.95–4.24 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Met2-α-H), 4.59 (t, 1H, 2′-H, $J$ = 4.7 Hz), 5.91 (d, 1H, 1′-H, $J$ = 5.5 Hz), 8.15 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 9.1, 14.7, 17.5, 29.9, 32.9, 45.7, 48.3, 54.5, 67.4, 70.9, 73.6, 82.7, 87.0, 119.0, 139.4, 149.7, 152.7, 156.1, 169.2, 174.8.

HRMS for $C_{18}H_{27}N_8O_8S_2$ [M−H]$^-$ calcd: 547.1394; found: 547.1394.

**4.1.3.21. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-methionyl)-vmethionyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 52).** Following the procedure used for the synthesis of **43**, **40** (250 mg, 0.43 mmol, 1.0 equiv) and *N*-Boc-L-methionine (116 mg, 0.51 mmol, 1.2 equiv) were reacted to yield 292 mg (0.40 mmol, 88%) of **52**.

HRMS for $C_{25}H_{39}N_8O_{10}S_3$ [M−H]$^-$ calcd: 707.1952; found: 707.1965.

**4.1.3.22. 5′-*O*-[*N*-(*N*-L-Methionyl)-L-methionyl]-sulfamoyladenosine (22).** Following the procedure used for the synthesis of **13**, **52** (287 mg, 0.35 mmol, 1.0 equiv) was deprotected to yield 130 mg (0.10 mmol, 21%) of **22**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.67–2.09 (m, 10H, 2 × Met-β-$H_2$, 2 × Met-*S*-$CH_3$), 2.32–2.6 (m, partly obscured by DMSO-$d_6$, 2 × Met-γ-$H_2$) 3.72 (t, 1H, Met1-α-H, $J$ = 6.3 Hz), 3.94-4.20 (m, 5H, 3′-H, 4′-H, 5′-$H_2$, Met2-α-H), 4.57 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.91 (d, 1H, 1′-H, $J$ = 6.0 Hz), 8.15 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 14.6, 14.7, 28.6, 29.9, 31.9, 32.7, 52.2, 54.5, 67.3, 70.9, 73.6, 82.7, 86.9, 119.0, 139.4, 149.7, 152.7, 156.1, 168.8, 174.8.

HRMS for $C_{20}H_{31}N_8O_8S_3$ [M−H]$^-$ calcd: 607.1427; found: 607.1438.

**4.1.3.23. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-prolyl)-L-methionyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 53).** Following the procedure used for the synthesis of **43**, **40** (265 mg, 0.46 mmol, 1.0 equiv) and *N*-Boc-L-proline (120 mg, 0.56 mmol, 1.2 equiv) were reacted to yield 294 mg (0.38 mmol, 83%) of **53**.

HRMS for $C_{25}H_{37}N_8O_{10}S_2$ [M−H]$^-$ calcd: 673.2074; found: 673.2045.

**4.1.3.24. 5′-*O*-[*N*-(*N* -L-Prolyl)-L-methionyl]-sulfamoyladenosine ($Et_3N$ salt, 23).** Following the procedure used for the synthesis of **13**, **53** (289 mg, 0.37 mmol, 1.0 equiv) was deprotected to yield 139 mg (0.21 mmol, 56%) of **23**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.18 (t, $Et_3NH^+$—$H_3$, $J$ = 7.3 Hz), 1.67–2.08 (m, 8H, Pro1-$\gamma-H_2$, Pro1-β-$H_A$, Met2-β-$H_2$, Met2-*S*-$CH_3$), 2.16–2.34 (m, 1H, Pro1-β-$H_B$), 2.44 (m, partly obscured by DMSO- $d_6$-peak, Met2-γ-H), 2.92-3.28 (m, partly obscured by HOD-peak, $Et_3NH^+$—$H_2$, Pro1-$\delta-H_2$), 3.93–4.25 (m, 6H, 3′-H, 4′-H, 5′-$H_2$, Met2-α-H, Pro1-α-H), 4.59 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.91 (d, 1H, 1′-H, $J$ = 6.1 Hz), 8.14 (s, 1H, 2-H), 8.38 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 8.6, 14.7, 23.6, 29.6, 29.9, 32.5, 45.5, 45.8, 54.8, 58.9, 67.4, 70.9, 73.6, 82.7, 87.0, 119.0, 139.4, 149.7, 152.7, 156.1, 167.8, 174.8.

HRMS for $C_{20}H_{29}N_8O_8S_2$ $[M-H]^-$ calcd: 573.1550; found: 573.1556.

**4.1.3.25. 5′-*O*-[*N*-[$N_\alpha$-(*N*-Boc-L-alanyl)-L-tryptophanyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 54).** Following the procedure used for the synthesis of **43**, **34a** (298 mg, 0.47 mmol, 1.0 equiv) and *N*-Boc-L-alanine (95 mg, 0.50 mmol, 1.1 equiv) were reacted to yield 180 mg (0.22 mmol, 48%) of **54**.

HRMS for $C_{29}H_{36}N_9O_{10}S$ $[M-H]^-$ calcd: 702.2306; found: 702.2316.

**4.1.3.26. 5′-*O*-[*N*-[$N_\alpha$-(L-Alanyl)-L-tryptophanyl]-sulfamoyl]-adenosine (24).** Following the procedure used for the synthesis of **13**, **54** (175 mg, 0.22 mmol, 1.0 equiv) was deprotected to yield 44 mg (0.07 mmol, 31%) of **24**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.31 (d, 3H, Ala-$H_3$, $J$ = 7.09 Hz), 2.92 (dd, 1H, Trp2-β-$H_A$, $J$ = 8.7 Hz, $J$ = 14.7 Hz), 3.25 (partly obscured by $H_2O$-peak, dd, Trp2-β-$H_B$, $J$ = 4.4 Hz, $J$ = 14.9 Hz), 3.67 (q, 1H, Ala1-α-H, $J$ = 6.9 Hz), 3.92–4.12 (m, 4H, 4′-H, 5′-$H_2$), 4.12-4.18 (m, 1H, Trp2-α-H), 4.34 (m, 1H, 3′-H), 4.61 (t, 1H, 2′-H, $J$ = 5.6 Hz), 5.93 (d, 1H, 1′-H, $J$ = 6.2 Hz), 6.95 (m, 1H, Trp-5-H), 7.03 (m, 1H, Trp-6-H), 7.12 (s, 1H, Trp-2-H), 7.30 (d, 1H, Trp-7H, $J$ = 8.0 Hz), 7.55 (d, 1H, Trp-4-H, $J$ = 7.7 Hz), 8.14 (s, 1H, 2-H), 8.41 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 18.0, 28.4, 48.5, 56.4, 67.3, 71.0, 73.7, 82.8, 86.9, 111.1, 111.2, 118.2, 118.5, 118.9, 120.7, 123.4, 127.7, 136.1, 139.4, 149.8, 152.7, 156.1, 169.7, 175.6.

HRMS for $C_{24}H_{30}N_9O_8S$ $[M+H]^+$ calcd: 604.1938; found: 604.1932.

**4.1.3.27. 5′-*O*-[*N*-[$N_\alpha$-(*N* -Boc-L-methionyl)-L-tryptophanyl]-sulfamoyl]adenosine ($Et_3N$ salt, 55).** Following the procedure used for the synthesis of **43**, **34a** (230 mg, 0.36 mmol, 1.0 equiv) and *N*-Boc-L-methionine (90 mg, 0.36 mmol, 1.0 equiv) were reacted to yield 193 mg (0.22 mmol, 61%) of **55**.

HRMS for $C_{31}H_{40}N_9O_{10}S_2$ $[M-H]^-$ calcd: 762.2340; found: 762.2331.

**4.1.3.28. 5′-*O*-[*N*-[$N_\alpha$-(L-Methionyl)-L-tryptophanyl]-sulfamoyl]-adenosine (25).** Following the procedure used for the synthesis of **13**, **55** (193 mg, 0.22 mmol, 1.0 equiv) was deprotected to yield 78 mg (0.12 mmol, 55%) of **25**.

$^1$H NMR (DMSO-$d_6$): $\delta$ 1.10 (t, $Et_3N-H_3$, $J$ = 7.2 Hz), 1.73–2.05 (m, 5H, Met2-β-$H_2$, Met2-*S*-$CH_3$), 2.83–3.04 (m, $Et_3NH^+-H_2$, Trp2-β-$H_A$), 3.26 (dd, 1H, Trp2-β-$H_B$, $J$ = 4.4 Hz, $J$ = 14.6 Hz), 3.60–3.66 (visible after addition of $D_2O$, m, 1H, Met1-α-H), 3.95–4.13 (m, 3H, 4′-H, 5′-$H_2$), 4.17 (m, 1H, Trp2-α-H), 4.35 (m, 1H, 3′-H), 4.61 (m, 1H, 2′-H), 5.92 (d, 1H, 1′-H, $J$ = 6.2 Hz), 6.94 (t, 1H, Trp-5H, $J$ = 7.5 Hz), 7.03 (t, 1H, Trp-6H, $J$ = 7.0 Hz), 7.12 (d, 1H, Trp-2H, $J$ = 2.1 Hz), 7.29 (d, 1H, Trp-7H, $J$ = 8.1 Hz), 7.53 (d, 1H, Trp-4-H, $J$ = 7.8 Hz), 8.14 (s, 1H, 2-H), 8.41 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 9.3, 14.5, 28.4, 28.5, 32.0, 52.2, 56.4, 67.3, 71.0, 73.7, 82.8, 86.9, 111.1, 111.2, 118.2, 118.4, 118.9, 120.7, 123.4, 127.7, 136.1, 139.4, 149.7, 152.7, 156.1, 168.8, 175.5.

HRMS for $C_{26}H_{33}N_9O_8S_2$ $[M-H]^-$ calcd: 662.1816; found: 662.1810.

**4.1.3.29. 5′-*O*-[*N*-[$N_\alpha$-(*N*-Boc-L-prolyl)-L-tryptophanyl]-sulfamoyl]-adenosine ($Et_3N$ salt, 56).** Following the procedure used for the synthesis of **43**, **34a** (270 mg, 0.43 mmol, 1.0 equiv) and *N*-Boc-L-proline (92 mg, 0.43 mmol, 1.0 equiv) were reacted to yield 240 mg (0.29 mmol, 67%) of **56**. HRMS for $C_{31}H_{38}N_9O_{10}S$ $[M-H]^-$ calcd: 728.2463; found: 728.2474.

**4.1.3.30. 5′-*O*-[*N*-[$N_\alpha$-(L-Prolyl)-L-tryptophanyl]-sulfamoyl]-adenosine (26).** Following the procedure used for the synthesis of **13**, **56** (235 mg, 0.28 mmol, 1.0 equiv) was deprotected to yield 132 mg (0.21 mmol, 75%) of **26**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.64–1.79 (m, 2H, Pro1-γ-$H_2$), 1.79–1.94 (m, 1H, Pro1-β-$H_A$), 2.08–2.24 (m, 1H, Pro1-β-$H_B$), 2.87–3.07 (m, 3H, Pro1-δ − $H_2$, Trp2-β-$H_A$), 3.26 (dd, partly obscured by $D_2O$-peak, Trp2-β-$H_B$, $J$ = 4.1 Hz, $J$ = 14.4 Hz), 3.89–4.20 (m, 5H, 4′-H, 5′-$H_2$, Pro1-α-H, Trp2-α-H), 4.34 (m, 1H, 3′-H), 4.62 (t, 1H, 2′-H, $J$ = 5.6 Hz), 5.92 (d, 1H, 1′-H, $J$ = 6.2 Hz), 6.92 (m, 1H, Trp-5H), 7.00 (m, 1H, Trp-6-H), 7.10 (s, 1H, Trp-2-H), 7.30 (d, 1H, Trp-7H, $J$ = 8.0 Hz), 7.53 (d, 1H, Trp-4-H, $J$ = 7.7 Hz), 8.14 (s, 1H, 2-H), 8.41 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 23.9, 28.3, 29.7, 46.0, 56.5, 59.2, 67.3, 71.0, 73.7, 82.8, 86.9, 111.0, 111.2, 118.2, 118.4, 119.0, 120.7, 123.3, 127.7, 136.0, 139.4, 149.8, 152.7, 156.1, 168.6, 175.4.

HRMS for $C_{26}H_{30}N_9O_8S$ $[M-H]^-$ calcd: 628.1938; found: 628.1934.

**4.1.3.31. 5′-*O*-[*N*-[$N_\alpha$-($N_\alpha$,*N*-Di-Boc-L-lysyl)-L-tryptophanyl]-sulfamoyl]adenosine ($Et_3N$ salt, 57).** Following the procedure used for the synthesis of **43**, **34a** (320 mg, 0.50 mmol, 1.0 equiv) and $N_\alpha$,*N*-di-Boc-L-lysine (173 mg, 0.50 mmol, 1.0 equiv) were reacted to yield 359 mg (0.37 mmol, 75%) of **57**.

HRMS for $C_{37}H_{51}N_{10}O_{12}S$ $[M-H]^-$ calcd: 859.3409; found: 859.3409.

**4.1.3.32. 5′-*O*-[*N* -($N_\alpha$-L-Lysyl)-L-tryptophanyl]-sulfamoyladenosine (27).** Following the procedure used for the synthesis of **16**, **57** (355 mg, 0.37 mmol, 1.0 equiv) was deprotected to yield 61 mg (0.09 mmol, 25%) of **27**.

$^1$H NMR (DMSO-$d_6$ + $D_2O$): $\delta$ 1.09–1.55 (m, 6H, Lys1-β-$H_2$, Lys1-γ-$H_2$, Lys1-δ-$H_2$), 2.60 (m, 2H, Lys1--$H_2$), 2.96 (dd, 1H, Trp2-β-$H_A$, $J$ = 7.4 Hz, $J$ = 14.7 Hz), 3.02-3.10 (m, 1H, Lys1-α-H), 3.20 (dd, Trp2-β-$H_B$, $J$ = 4.7 Hz, $J$ = 14.6 Hz), 3.94–4.13 (m, 3H, 4′-H,5′-$H_2$), 4.13–4.19 (m, 1H, Trp2-α-H), 4.31 (m, 1H, 3′-H), 4.59 (t, 1H, 2′-H, $J$ = 5.5 Hz), 5.92 (d, 1H, 1′-H, $J$ = 6.0 Hz), 6.92 (m, 1H, Trp2-5-H), 7.01 (m, 1H, Trp2-6-H), 7.09 (s, 1H, Trp2-2-H), 7.28 (d, 1H, Trp-H, $J$ = 8.1 Hz), 7.49 (d, 1H, Trp-4-H, $J$ = 7.7 Hz), 8.14 (s, 1H, 2-H), 8.43 (s, 1H, 8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 22.0, 28.5, 28.7, 34.5, 54.5, 55.7, 67.3, 70.9, 73.8, 82.7, 86.9, 111.0, 111.1, 118.0, 118.6, 118.9, 120.6, 123.4, 128.0, 135.9, 139.4, 149.7, 152.7, 156.1, 174.2, 176.3.

HRMS for $C_{27}H_{35}N_{10}O_8S$ $[M-H]^-$ calcd: 659.2360; found: 659.2363.

**4.1.3.33. 5′-*O*-[*N*-[$N_\alpha$-(*N* -Boc-L-glutamyl-γ-*O*-*tert*-butyl)-L-tryptophanyl]-sulfamoyl]adenosine ($Et_3N$ salt, 58).** Following the procedure used for the synthesis of **43**, **34a** (360 mg, 0.57 mmol, 1.0 equiv) and *N*-Boc-L-glutamic acid γ-*O*-*tert*-butyl ester (172 mg, 0.57 mmol, 1.0 equiv) were reacted to yield 359 mg (0.39 mmol, 69%) of **58**.

HRMS for $C_{35}H_{46}N_9O_{12}S$ $[M-H]^-$ calcd: 816.2987; found: 816.2987.

**4.1.3.34. 5′-*O*-[$N_\alpha$-(*N*-L-Glutamyl)-L-tryptophanyl]-sulfamoyladenosine ($Et_3N$ salt, 28).** Following the procedure used for the synthesis of **17**, **58** (339 mg, 0.37 mmol, 1.0 equiv) was deprotected to yield 189 mg (0.23 mmol, 61%) of **28**.

$^1$H NMR (DMSO-$d_6$): $\delta$ 1.14 (t, $Et_3N-H_3$, $J$ = 7.3 Hz), 1.74–2.03 (m, 2H, Glu1-β-$H_2$), 2.38 (t, 1H, Glu1-γ-$H_2$, $J$ = 7.5), 2.78–3.03 (m, $Et_3N-H_2$, Trp2-β-$H_A$), 3.26 (dd, 1H, Trp2-β-$H_B$, $J$ = 4.2 Hz, 14.6 Hz), 3.57 (t, 1H, Glu1-α-H, $J$ = 6.0 Hz), 3.95–4.13 (m, 3H, 4′-H, 5′-$H_2$), 4.13–4.21 (m, 1H, Trp2-α-H), 4.35 (dt, 1H, 3′-H, $J$ = 4.3 Hz, 8.4 Hz), 4.61 (t, 1H, 2′-H, $J$ = 5.6 Hz), 5.92 (d, 1H, 1′-H, $J$ = 6.2 Hz), 6.94 and 7.03 (2 × m, 2H, Trp-5-H and Trp-6-H), 7.13 (d, 1H, Trp-2-H, $J$ = 2.2 Hz), 7.26 (br s, 2H, Ade-$NH_2$), 7.30 (m, 1H, Trp-7-H, $J$ = 7.9 Hz), 7.55 (d, 1H, Trp-4-H, $J$ = 7.7 Hz), 8.13 (s, 1H, 2-H), 8.41 (s, 1H, 8-H), 10.75 (d, 1H, indole-NH, $J$ = 2.1 Hz).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 9.1, 27.8, 28.4, 30.9, 45.5, 52.4, 56.3, 67.4, 71.0, 73.8, 82.9, 86.9, 111.1, 111.2, 118.2, 118.4, 118.9, 120.7, 123.4, 127.7, 136.1, 139.4, 149.8, 152.7, 156.1, 169.3, 174.6, 175.6.

HRMS for $C_{26}H_{30}N_9O_{10}S$ $[M-H]^-$ calcd: 660.1837; found: 660.1833.

**4.1.3.35. 5′-*O*-[*N*-[*N*-(*N*-Boc-L-Methionyl)-L-aspartyl]-sulfamoyl] adenosine ($Et_3N$ salt, 59).** Following the procedure used for the synthesis of **43**, **41** (80 mg, 0.17 mmol, 1.0 equiv) and *N*-Boc-L-methionine (48 mg, 0.19 mmol, 1.1 equiv) were reacted and purified by silica gel chromatography ($Et_3N$ 1%, MeOH 5→30% in $CH_2Cl_2$) to yield 130 mg (0.16 mmol, 96%) of **29**.

HRMS for $C_{24}H_{37}N_8O_{12}S_2$ $[M+H]^+$ calcd: 693.1972; found: 693.19536.

**4.1.3.36. 5′-*O*-[*N*-(*N*-L-Methionyl)-L-aspartyl]-sulfamoyladenosine ($Et_3N$ salt, 29).** Compound **59** (130 mg, 0.16 mmol, 1.0 equiv) was dissolved in 5 mL TFA/$H_2O$ (5:2, v/v) and stirred for 1.5 h at rt. Next, the volatiles were evaporated and coevaporated twice with EtOH and once with $Et_3N$ (0.5 mL). After evaporation of the volatiles, the residue was purified by silica gel chromatography ($Et_3N$ 1%, MeOH 20→60% in $CHCl_3$) and further purified by ion exchange chromatography (DEAE-cellulose column, triethylammonium bicarbonate 0→0.6 M in water) to yield 45 mg (0.065 mmol, 41%) of **29**.

$^1$H NMR (DMSO-$d_6$): $\delta$ 1.03 (t, 9H, $Et_3N^+-H_3$, $J$ = 7.2 Hz), 1.67–2.08 (m, 5H, Met1-β-$H_2$, Met1-*S*-$H_3$), 2.40–2.61 (obscured by DMSO-$d_6$ peak, β-$H_2$), 2.61–2.80 (m, β-$H_2$, $Et_3NH^+-H_2$), 2.86 (q, 6H, $Et_3N$-$H_2$, $J$ = 7.2 Hz), 3.48 (t, 1H, Met1-α-H, $J$ = 6.0 Hz), 3.96–4.12 (m, 3H, 4′-H, 5′-$H_2$), 4.12–4.19 (m, 1H, 3′-H), 4.35 (dd, 1H, Asp2-α-H, $J$ = 7.2 Hz, 13.1 Hz), 4.58 (t, 1H, 2′-H, $J$ = 5.4 Hz), 5.91 (d, 1H, 1′-H, $J$ = 6.1 Hz), 7.26 (br s, 2H, Ade-$NH_2$, $D_2O$-exchangeable), 8.15 (s, 1H, Ade-2-H), 8.20 (d, 1H, Asp-NH, $J$ = 8.0 Hz), 8.39 (s, 1H, Ade-8-H).

$^{13}$C NMR (DMSO-$d_6$): $\delta$ 11.3, 14.6, 29.5, 34.2, 38.6, 45.7, 51.9, 53.6, 67.3, 70.9, 73.7, 82.7, 86.8, 118.9, 139.3, 149.7 152.7, 156.1, 172.7, 172.8, 174.7.

HRMS for $C_{19}H_{27}N_8O_{10}S_2$ $[M-H]^-$ calcd 591.1292; found 591.1281.

Basic data analysis was done in Microsoft Excel 2003 (Microsoft Corporation, Redmond, WA, USA). Where applicable, data are represented as the mean ± SD. Testing for significant differences between inhibition zones was done using the unpaired student *t*-tests were done in Graphpad Prism 4.03 (GRAPHPAD Software, 200). *P*-Values $\leqslant$0.05 were considered statistically significant. INSTANT JCHEM was used for structure database management, search and prediction, INSTANT JCHEM 2.3, 2008, CHEMAXON (http://www.chemaxon.com). Chemdraw was used for chemical structure and reaction drawing, and for calculating exact masses (Chemdraw 11, CambridgeSoft Corporation, Cambridge, MA, USA).

### 4.2. Biological evaluation

Liquid Mueller–Hinton Agar (0.75%) was mixed with bacterial suspension (29:1 v/v) at rt. Plates were prepared by adding 4 mL of this suspension to petri plates containing 7 mL of solidified Mueller–Hinton Agar (1.5%). Immediately after solidification of the agar, 5 μL drops containing investigational compound in $H_2O$ milliQ were added and allowed to dry. After incubation for 16 h at 37 °C, the inhibition zones were measured. These evaluations were performed in triplicate for each compound–concentration combination.

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## Supplementary data

The following supplementary data are available: Table S1, summarizing the results from the initial antibacterial screening of all 20 analogues of 5′-*O*-(*N*-L-aminoacyl)-sulfamoyladenosine. Table S2, summarizing the results from the antibacterial evaluation of the new 5′-*O*-(*N*-L-dipeptidyl)-sulfamoyladenosine **12**–**29** (Fig. 4) and selected reference compounds with lower doses of compounds (6 nmol). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.11.054.

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