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Investigation, optimization and synthesis of sulfamoyloxy-linked aminoacyl-AMP analogues

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ABSTRACT

Aminoacyl-tRNA synthetases (aaRSs) constitute a family of enzymes that transfer amino acids to their corresponding tRNA molecules to form aminoacyl-tRNAs and have been validated as potential drug targets. Sulfamoyloxy-linked aminoacyl-AMP analogues are potent inhibitors of aaRSs. In this article, we report the synthesis of several new sulfamoyl analogues of aa-AMP that up to now have been difficult or even impossible to prepare with current synthetic strategies. The developed synthetic strategy relies on performing the synthesis under neutral conditions followed by global deprotection using catalytic hydrogenation affording the desired 5′-*O*-(*N*-aminoacyl)sulfamoyladenosine compounds.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) are enzymes essential for the viability of all living organisms because of their role in protein biosynthesis.[1] These enzymes are responsible for the ATP dependent coupling of the correct amino acid to its cognate tRNA through a two-step process (Scheme 1). The formation of an enzyme-bound aminoacyl-adenylate monophosphate (aa-AMP) is followed by the transfer of this activated amino acid to either the 2′-or 3′-hydroxy group of the adenosine moiety of tRNA.

$$\text{Amino acid} + \text{ATP} \rightarrow \text{aa-AMP} + \text{PPi}$$
$$\text{aa-AMP} + \text{tRNA} \rightarrow \text{aa-tRNA} + \text{AMP}$$

Scheme 1. Schematic overview of the ATP dependent two-step process catalysed by aaRSs resulting in the esterification of tRNA by its cognate amino acid.

AaRSs have emerged as an interesting target class for the development of anti-infective drugs. When aaRS is inhibited, the corresponding tRNA is not loaded and is therefore unavailable for translation. Thus, inhibition of aaRS will halt protein synthesis and attenuate microorganism growth.[2–4] Various chemical structures that inhibit aaRSs have been identified.[5] In general, these inhibitors are based on the natural synthetase substrates and reaction intermediates, or have been identified by screening of compound libraries. For example, the natural product mupirocin is used as an antibacterial drug in clinical applications (IleRS inhibitor),[6] and REP8839, a bacterial MetRS inhibitor, is currently under clinical investigation.[7] Recently, an antifungal agent that inactivates fungal LeuRS was reported[8] and has now entered clinical trials. Furthermore, aaRSs are considered as attractive drug targets in disease pathogens such as mycobacteria, protozoans and helminths.[9]

The design of most aa-AMP analogues reported so far has been based on replacing the labile acyl-phosphate linkage of the intermediate with stable bioisosteres such as alkylphosphates,[10–12] esters,[13–15] amides,[13] hydroxamates,[13–15] sulfamates,[10,11,16–19] sulfamides,[11] *N*-alkoxysulfamides[20] and *N*-hydroxysulfamides.[20] We have designed and synthesised several non-hydrolysable sulfamoyl analogues of aa-AMP that have been used by collaborators in structural studies on a number of aaRSs.[21–23] These studies are possible as the analogue binding leads to stabilisation of the active site, enables identification of the specific amino acid binding pocket and contributes to the characterisation of the conformational changes associated with aminoacyl-adenylate formation. Such studies have been carried out on the asparaginyl-[21,22] and leucyl[23]-adenylate analogues leading to significant new results.

Although sulfamoyl based analogues of aa-AMP are useful for structural and biochemical studies of aaRSs and as potential lead compounds for drug development, their synthesis is hampered by

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several limitations. The overall yields are highly dependent on the amino acid and the protecting groups used in the synthesis and consequently some sulfamoylated analogues of aa-AMP are difficult to attain.

Herein, we report a novel synthetic strategy enabling the efficient synthesis of several new sulfamoyl analogues of aa-AMP that have been difficult to prepare up to now. The reported procedure relies on an orthogonal protecting group strategy and global deprotection under neutral conditions.

2. Results and discussion

2.1. Protective group strategy

A commonly used intermediate for the preparation of sulfamoylated analogues of aa-AMP is compound **1** (Fig 1) prepared from 2′,3′-*O*-isopropylidene protected adenosine by sulfamoylation.[24] However, both **1** as well as derivatives with the general structure **2** are prone to cyclonucleoside formation (**3**) (Fig. 1) resulting in low yields of the desired products.[25,26] A protecting group strategy or synthetic procedure that minimizes or eliminates this side-reaction is necessary to be able to increase the yields in the synthesis of sulfamoylated analogues of aa-AMP.

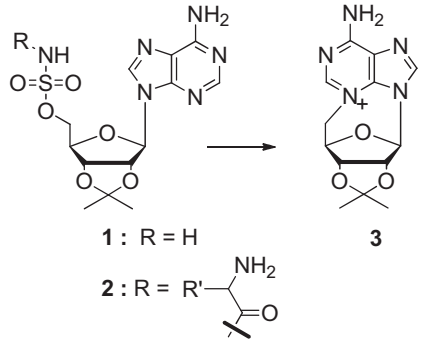


Fig. 1. Compounds **1** and **2** are prone to cyclonucleoside (**3**) formation.

In our studies we also aimed to prepare sulfamoylated aa-AMP analogues containing non-proteinogenic amino acids, e.g., homoserine and homocysteine. However, such aa-AMPs can easily undergo intramolecular cyclisation reactions generating lactone/thiolactone products (Fig. 2). It is believed that LysRS edits homocysteine by such reactions (**4**, X=S, Q=O, Z=OH, n=1).[27] In principle, this reaction can occur with all derivatives having an amino acid side chain with appropriate length (n=1, 2 or 3) in combination with a nucleophilic heteroatom at the end of the side chain (X=NH, O or S). Furthermore, the cyclisation reaction is most likely promoted by basic and acidic conditions, respectively. Consequently, only protecting groups that are removed under neutral conditions can be applied in the protecting group strategy to avoid the formation of the cyclic byproducts (**6**).

The synthesis of compounds **1** and **2** does not require protection of the exocyclic amino function of the adenine moiety,[24] but these compounds are rather polar and the recovery after column chromatography can be low. Although the introduction of a protecting group for this amino function will add additional steps to the synthesis sequence, it will also result in more lipophilic intermediates, which could easily be purified by flash chromatography.

Considering these aspects we designed a general and convergent protecting group strategy for the synthesis of sulfamoylated aa-AMPs. We reasoned that the combination of a benzyl carbamate (Cbz) group for protection of the exocyclic amino function of the adenine moiety as well as the amino function of the amino acids,

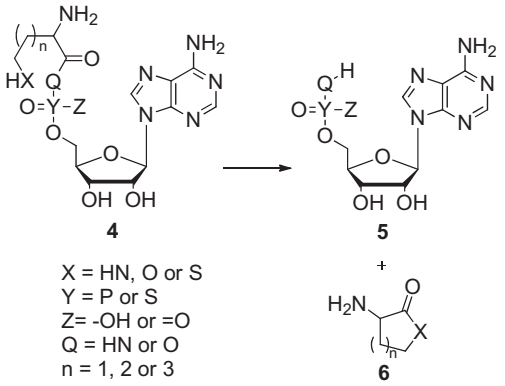
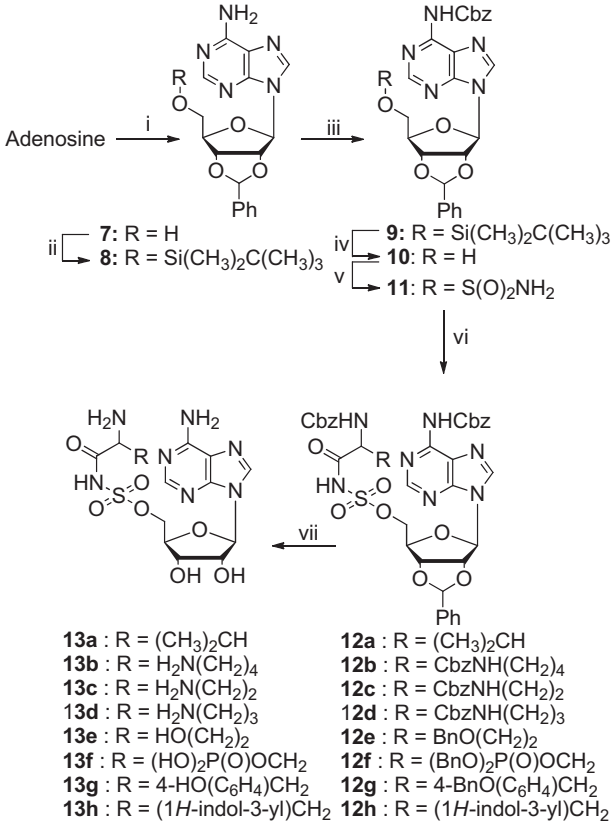


Fig. 2. Lysyl-tRNA synthetase edits homocysteine by converting it into homocysteine thiolactone. (X=S, Y=P, Z=OH, Q=O, n=1). A similar intramolecular reaction could potentially occur with synthetic derivatives of aa-AMP, derivatives (Q=NH).

a benzylidene acetal protection group for the 2′- and 3′-hydroxy functions of adenosine and a Cbz or benzyl (Bn) protective group of the hetero functionality of the amino acid side chain would be an excellent choice for the preparation of aa-AMP analogues. This combination of protecting groups would enable global deprotection using catalytic hydrogenation.

2.2. Synthesis

The synthesis of the target compounds is illustrated in Scheme 2.



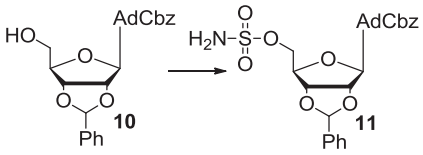
Scheme 2. Synthesis of sulfamoylated aa-AMP analogues. Reagents and conditions: (i) $ZnCl_2$ (5 equiv), benzaldehyde, rt, 72 h, 92%; (ii) TBDMS-Cl (3 equiv), imidazole (7 equiv), pyridine, rt, 24 h, 94%; (iii) Rapoport's reagent (3.4 equiv), DCM, rt, 24 h, 96%; (iv) TBAF (4 equiv), THF, rt, 24 h, 95%; (v) sulfamoyl chloride (2.2 equiv), DMAP (2.3 equiv), DCM, 0 °C→rt, 24 h, 96%; (vi) amino acid (3 equiv), PS-DCC (4 equiv), DMAP (3 equiv), DCM/DMF, rt, 24 h, 49–85%; (vii) continuous-flow hydrogenation, water/2-propanol (5:95 v/v), flow rate=1 mL/min, 50 bar, 70 °C, 10% Pd/C CatCart, 30×4 mm, 120 min, 20–78%.

Adenosine was first treated with benzaldehyde in the presence of zinc chloride (5 equiv) to protect the 2′-and the 3′-hydroxyl groups yielding **7** in 92% after column chromatography. Subsequent 5′-*O*-*tert*-butyldimethylsilyl (TBDMS) protection using TBDMS–Cl and imidazole in pyridine furnished compound **8** in 94% yield after purification by flash chromatography. The exocyclic amino group was protected as a benzyl carbamate using Rapoport's reagent (1-benzyloxycarbonyl-3-ethylimidazolium tetrafluoroborate).[28] A solution of **8** in DCM was added dropwise to the freshly prepared acylimidazolium salt in DCM and the mixture was stirred overnight. Compound **9** was obtained in 96% yield after purification by flash chromatography. Subsequent removal of the 5′-*O*-silyl protective group using tetrabutylammonium fluoride (TBAF) proceeded smoothly yielding **10** in 95% yield after flash chromatography.

2.3. Sulfamoylation

Several protocols for sulfamoylation of 2′,3′-*O*-isopropylidene protected adenosine have been reported in the literature including sulfamoyl chloride and NaH in DMF,[29] sulfamoyl chloride and DBU,[30] or sulfamoyl chloride in DMA.[31] We decided to test various bases differing in strength and in steric hindrance (Table 1). Strong bases such as NaH (entry 1) and DBU (entry 2) gave only low yields of **11**, partly due to decomposition of sulfamoyl chloride.

Table 1
Different bases used for sulfamoylation of **10**[a]



Entry	Base	Yield (%)
1	NaH	13
2	DBU	17
3	TEA	—
4	DIPEA	—
5	DMA[b]	41
6	DMAP[c]	96

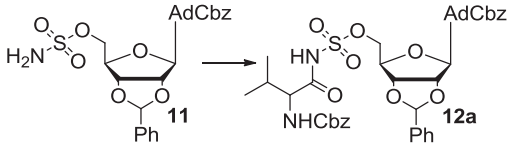
[a] Reaction conditions: **10** (0.15 mmol), sulfamoyl chloride (1 equiv), DCM, 4 h, rt, inert atmosphere.
[b] DMA as solvent, 4 h, rt, inert atmosphere.
[c] Sulfamoyl chloride (2.2 equiv), DMAP (2.3 equiv), 0 °C. 4 h, inert atmosphere.

The use of weaker but more sterically hindered amine bases, such as TEA (entry 3) or DIPEA (entry 4), resulted in only trace amounts of the target product. The use of DMA as solvent without the addition of any base has been reported to give sulfamoylation of alcohols in high yields.[31] Unfortunately, in our hands this method only resulted in a moderate yield of product (entry 5). Another known method comprised the reaction of *N*-(*tert*-butoxycarbonyl) sulfamoyl chloride with DMAP to form a non-moisture sensitive and stable azanide salt that allows sulfamoylation of amines under very mild conditions.[32] We therefore tested DMAP as a base (entry 6) in the sulfamoylation reaction and obtained **11** in an excellent yield of 96% after purification by flash chromatography.

2.4. Amino acid coupling reaction

A common method for coupling protected amino acids to sulfamoyl moieties is to use *N*-(*tert*-butyl carbamate) (Boc) protected *N*-hydroxysuccinimide activated amino acids in the presence of DBU.[24,26] However, not all amino acids are commercially available as hydroxysuccinimide esters and their preparation is not always straightforward. We therefore decided to test a range of different coupling reagents, including those that can be used for in situ generation of the required active ester (Table 2).

Table 2
Different coupling mixtures used for amide bond formation[a]



Entry	Coupling mixture	Conversion[c]
1	aa-OSu/DBU	Moderate
2	aa-Pfp/HOBt	Low
3	aa-OH/TBTU/HOBt	Low
4	aa-OH/PyBrOP	Low
5	aa-OH/HATU/DIPEA	Moderate
6	aa-OH/EDC/HOBt	Moderate
7	aa-OH/DCC/DMAP	High
8	aa-OH/PS-IIDQ[b]	Moderate
9	aa-OH/PS-EDC/HOBt[b]	Moderate
10	aa-OH/PS-DCC/DMAP[b]	High

[a] Reaction conditions: **11** (0.15 mmol), amino acid (1 equiv), DMF, overnight, rt, inert atmosphere.
[b] Coupling agent polymer bound to polystyrene.
[c] Determined by LCMS, low=10–40%, moderate=40–65%, high=65–99%.

N-Hydroxysuccinimide activated valine in combination with DBU (entry 1) gave moderate conversion of **11** to **12a**, while the pentafluorophenyl activated valine gave hardly any product formation at all (entry 2). Other commonly used coupling reagents like TBTU/HOBt, PyBrOP and HATU (entries 3–5) gave only low to moderate conversion of the starting material to the product. However, the reaction with HATU stopped after only 1 h, probably due to the poor stability of the coupling reagent. The carbodiimide-based coupling reagents EDC (entry 6) and DCC (entry 7) gave moderate to high conversation. Although DCC resulted in high conversion of the starting material, the byproduct *N*,*N*′-dicyclohexylurea is often difficult to remove from the reaction mixture. We therefore decided to test polymer bound DCC for the conversion of **11** to **12a**. We also included polymer bound EDC as well as polymer bound IIDQ in the tests. The polymer bound coupling reagents were first preincubated with the Cbz-protected valine for 1 h before the resin was washed and suspended in dry DMF and mixed with a solution of **11**. Polymer bound DCC gave excellent conversion (entry 10), while the use of PS-EDC (entry 9) or PS-IIDQ (entry 8) resulted in only moderate conversion.

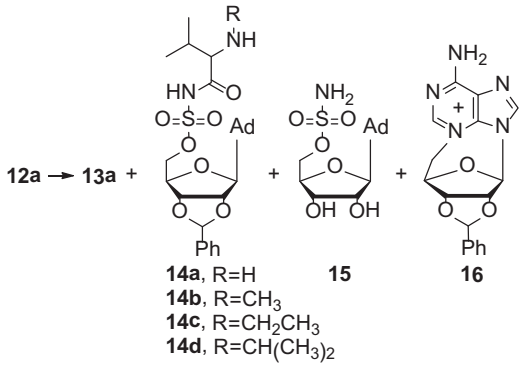
Compounds **12a**–**h** were synthesised using PS-DCC/DMAP and could be obtained in 49–85% yield after column chromatography and preparative HPLC. In the ^{1}H and ^{13}C NMR spectra doubled signals were observed. From variable temperature ^{1}H and ^{13}C NMR experiments it could be concluded that **12a**–**h** adopts two different conformations with a high activation energy for the interconversion.[33]

2.5. Deprotection by catalytic hydrogenation

We decided to use catalytic hydrogenation for deprotection of **12a**–**h** using a continuous flow, high pressure device (H-cube®) that allowed easy testing of different types of catalysts and variations of reactions conditions such as hydrogen pressure, solvents and temperature (Table 3).

The initial tests were carried out using 5% Pd/C, MeOH as solvent at 1 bar, rt and a reaction time of 40 min (entry 1). LCMS analysis of the reaction mixture showed 10–20% conversion of **12a** to the

Table 3
Different reaction conditions used for hydrogenolytic deprotection of **12a** (0.015 mmol)



Entry	Catalyst	Solvent	Pressure (bar)	Temp (°C)	Time (min)	Conversion[b] **12a→13a**
1	5% Pd/C	MeOH	1	rt	40	Low
2	10% Pd/C	EtOH	1	rt	40	Low
3	10% Pd/C	EtOH	1	rt	80	Low
4	10% Pd/C	EtOH	10	rt	40	Low
5	10% Pd/C	EtOH[a]	30	30	40	Moderate
6	10% Pd/C	EtOH[a]	50	50	80	Moderate
7	10% Pd/C	2-PrOH[a]	50	50	80	Moderate
8	10% Pd/C	2-PrOH[a]	50	70	120	High
9	10% Pd/C	tBuOH[a]	50	70	120	Low
10	20% $Pd(OH)_2$	2-PrOH[a]	50	70	120	Moderate

[a] With 5% water present.
[b] Determined by LCMS, low=10–40%, moderate=40–65%, high=65–99%.

desired product **13a**. Unfortunately, the major product formed in the reaction was intermediate **14a** (50%). Traces of product (<5%) with a mass indicating deprotection of one of the two Cbz groups were also detected. Prolonged reaction time, higher pressure or temperature did not improve the yield of **13a** significantly (data not shown).

In order to accelerate the reaction and to increase the solubility of **12a**, 10% Pd/C was tested in combination with EtOH as solvent (entry 2). LCMS analysis of the reaction mixture after 40 min showed a conversion of approximately 65% of starting material to **14a**. In addition, the same byproduct as identified in entry 1 (one Cbz group removed) was obtained in comparable amount. The desired product **13a** was detected in 25–35% yield by LCMS.

Different pressures, temperatures and reaction times (entries 3–6) were investigated to improve the conversion of **12a** to the target compound **13a**. However, increasing temperature and pressure also leads to formation of an additional byproduct **14c**. The formation of the latter product can be explained by dehydrogenation of ethanol to acetaldehyde by the palladium catalyst and subsequent reaction of the amino group of the valinyl moiety with the aldehyde to form a Schiff base, which was reduced to afford an *N*-ethylated amine.[34] The corresponding byproduct **14b** was observed when the hydrogenation was performed in MeOH at high pressure and high temperature (data not shown). To reduce the amount of N-alkylation, ethanol containing 5% water was used as solvent (entry 5) and the reaction time was extended to 80 min to increase the conversion of the starting material. Although more than 80% of the starting material had been consumed, compound **14a** was the major product and only trace amounts of **13a** were detected. Furthermore, the water did not supress the N-alkylation of the valinyl moiety to give **14c**. However, at 50 bar, 50 °C and a reaction time of 80 min additional byproducts (**15** and **16**, <20%) were detected, in addition to **14c**. Running the hydrogenation reaction in 2-propanol (entries 7 and 8) reduced the amount of N-alkylation to give **14d**, but did not suppress the formation completely. However, running the reaction at 50 bar and 70 °C for 120 min resulted in full conversion of **12a** to **13a** with only trace amounts of **14a**, **14d**, **15** and **16**. Using *t*-BuOH as solvent (entry 9) or $Pd(OH)_2$ as catalyst (entry 10) resulted in much slower conversion of the starting material and an incomplete reaction.

Based on these results we decided to carry out the deprotection of **12a**–**h** using 2-propanol as solvent, 70 °C, 50 bar and a reaction time of 120 min. Hydrogenolytic deprotection of **12a** gave **13a** in 58% yield, with trace amounts of the byproducts **14d**, **15**, **16** and adenosine were also detected. While deprotection of **12b** resulted in 36% of **13b**, byproduct **15** was also formed in approximately 35% yield, in addition to trace amounts of adenosine and **15**. The low yield of **13b** was probably due to the amino acid side chain having a favourable length (n=3) to allow the undesired lactam formation described in Fig. 2. We reasoned that the relatively high pressure and temperature applied during the hydrogenation could favour byproduct **16** and adenosine formation (Table 3). Hydrogenation of **12c** formed the desired product **13c** in 78% yield with only traces of byproduct. The deprotection of **12d** resulted mainly in the formation of byproduct **15** (approximately 60%) while **13d** was only isolated in 20% yield. The low yield of **13d** (where n=2) was probably due to lactam formation as described for deprotection of **12b**. Hydrogenolysis of **12e** also resulted in the byproduct **15** as the major product with **13e** (where n=2) isolated in 40% yield due to lactone formation. Deprotection of **12f**, **12g** and **12h** resulted in isolation of **13f** in 77%, **13g** in 66% and **13h** in 60% yield. Traces of byproduct **16** could be observed and the considerably better yields were probably due to the amino acid side chains in **12f**, **12g** and **12h** not able to perform the undesired intramolecular cyclisation reaction (Fig. 2). The synthesis of compounds **13g** and **13h** has been published earlier. Even though our synthetic strategy requires more steps then methods reported in the literature to attain the target compounds, the overall yields are comparable for **13h**[35] and even higher for **13g**.[11]

3. Conclusions

We have developed a convenient and convergent protecting group strategy for the synthesis of sulfamoylated aa-AMP analogues. The selected protecting groups can be incorporated in high yields, they withstand the applied reaction conditions, and they can be removed by hydrogenolysis in a single step under neutral conditions to avoid product decomposition. The developed synthetic protocol should be useful for the preparation of a range of natural and non-proteinogenic amino acid sulfamoyl adenolates. Careful monitoring and optimization of the reaction conditions allow the formation of most of these byproducts to be kept at a minimum. The synthesis protocol leading to the target compounds requires more steps than published methods (introduction and removal of the protecting groups) but one of the advantages is a simplified purification (due to increased lipophilicity). The overall isolated yields are comparable and even higher than those in reported methods.[11,35] Our method makes it possible to prepare a range of new and novel sulfamoyloxy-linked aminoacyl-AMP analogues.

4. Experimental section

4.1. General

All commercial chemicals were used without prior purification. Dichloromethane and pyridine were distilled from calcium hydride. Tetrahydrofuran was distilled from sodium/benzophenone. All reactions were monitored by TLC (Merck silica gel 60 F_{254}) and analysed under UV (254 nm). Column chromatography was performed

by manual flash chromatography (wet packed silica, 0.04–0.063 mm) or by automated column chromatography on a Biotage SP-4 instrument using pre-packed silica columns. Preparative high performance liquid chromatography (HPLC) was carried out on a Waters 600 controller connected to a Waters 2487 Dual λ Absorbance detector using a Atlantis® Prep T3 5 μm C-18 (250×19 mm) column unless otherwise stated. Microwave reactions were performed in a Biotage Initiator reactor with fixed hold time. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were obtained from a JEOL JNM-EX 400 spectrometer. IR spectra were acquired on a ChiralIR-2X Biotools instrument. Measurements of the optical rotations were performed on a Perkin Elmer 341LC polarimeter. Melting point determined on a Mettler FP800 apparatus without corrections.

4.2. 2′,3′-*O*-Benzylideneadenosine (7)

Adenosine (5.0 g, 18.8 mmol) and dry $ZnCl_2$ (10.5 g, 93.6 mmol) was suspended in 30 mL freshly distilled benzaldehyde (100 °C, 12 mbar). The reaction mixture was stirred at rt for 72 h under N_2 atmosphere. The solvent was removed under reduced pressure and the mixture was co-distilled with toluene. The yellow glue-like residue was suspended in ethyl acetate (100 mL) and washed with saturated $NaHCO_3$ solution (30 mL). The aqueous layer was extracted with ethyl acetate (3×250 mL). The combined organic layers were washed with water (200 mL), dried over $MgSO_4$, filtered and the solvent removed under reduced pressure and the residue was co-distilled with toluene. The crude product was dissolved in DCM/MeOH, silica gel (10 g) was added and the volatiles were removed under reduced pressure. The adsorbed residue was purified by column chromatography (stepwise gradient from 0–10% v/v MeOH in $CHCl_3$), yielding **7** (6.1 g, 92%) as white crystals, mp 201–203 °C (lit. mp 204 °C[36]). R_f=0.3 (MeOH/DCM, 1:10, v/v). NMR data were in agreement with published data.[37]

4.3. 2′,3′-*O*-Benzylidene-5′-*O*-(*tert*-butyldimethylsilyl) adenosine (8)

Compound **7** (3.3 g, 7.6 mmol), imidazole (4.6 g, 67.6 mmol) and TBDMS–Cl (5.0 g, 33.4 mmol) were stirred in dry pyridine (30 mL) for 24 h at rt under N_2 atmosphere. The reaction was quenched by the addition of ice-water (100 mL). The aqueous solution was extracted with $CHCl_3$ (3×100 mL), the organic phases were combined and the solvent was removed under reduced pressure and co-distilled with toluene. Aqueous acetic acid (40 mL, 80%) was added to the residue and the mixture was stirred for 20 min at rt. The reaction was quenched by the addition of ice-water (100 mL) and the aqueous solution extracted with $CHCl_3$ (3×100 mL). The organic layers were pooled and solid $NaHCO_3$ (approximately 10 g) was added, the suspension was stirred for 45 min and washed with water (2×100 mL), the organic layer was separated, dried over $MgSO_4$, and filtered before the solvent was removed under reduced pressure and the residue was co-distilled with toluene. The crude product was purified by automated flash chromatography (stepwise gradient, 2–20% v/v, MeOH in DCM), yielding **8** as a white foam (4.1 g, 94% yield). R_f=0.4 (MeOH/DCM, 1:10, v/v). ^{1}H NMR ($CDCl_3$) δ 0.01 (6H, s), 0.08 (9H, s), 3.75–3.90 (2H, m), 4.59–4.61 (1H, m), 5.09 (1H, dd, *J* 6.2 and 2.2 Hz), 5.52 (1H, dd, *J* 6.6 and 2.6 Hz), 5.75 (2H, br s), 6.04 (1H, s), 6.30 (1H, d, *J* 2.6 Hz), 7.43–7.44 (3H, m), 7.55–7.60 (2H, m), 8.05 (1H, s), 8.37 (1H, s). ^{13}C NMR ($CDCl_3$) δ 5.4, 18.4, 25.9, 63.6, 83.0, 85.4, 87.4, 91.4, 107.6, 120.1, 126.8, 127.0, 128.6, 128.6, 130.1, 135.9, 139.5, 149.4, 153.1, 155.9. Anal. Calcd for $C_{23}H_{31}N_5O_4Si$: 58.83 (C); 6.65 (H); 14.91 (N). Found: 58.81 (C); 6.71 (H); 14.83 (N).

4.4. 6-*N*-Benzyloxycarbonyl-2′,3′-*O*-benzylidene-5′-*O*-(*tert*-butyldimethylsilyl)adenosine (9)

A solution of **8** (5.5 g, 11.7 mmol) in dry DCM (50 mL) was added dropwise to a stirred solution of freshly prepared Rapoport's reagent (1-(benzyloxycarbonyl)-3-ethylimidazolium tetrafluoroborate) in DCM (100 mL, 0.4 M) at rt under N_2 atmosphere. After 24 h, the reaction mixture was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (50 mL) and diluted with DCM (50 mL). The organic layer was separated, washed with water (20 mL), dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (stepwise gradient from 2 to 20% v/v MeOH in DCM), yielding compound **9** as a white foam (6.8 g, 96%). R_f=0.7 (MeOH/DCM, 1:10, v/v). ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s), 0.81 (9H, s), 3.74–3.82 (2H, m), 4.61 (1H, br s), 5.06 (1H dd, *J* 6.2 and 1.5 Hz), 5.23 (2H, br s), 5.49 (1H, dd, *J* 6.2 and 1.8 Hz), 6.02 (1H, s), 6.23 (1H, d, *J* 2.20 Hz), 7.31–7.61 (10H, m), 8.14 (1H, s), 8.79 (1H, s), 9.61 (1H, s). ^{13}C NMR ($CDCl_3$) δ 5.6, 18.2, 25.8, 63.4, 67.7, 69.7, 76.9, 77.5, 82.9, 85.2, 87.3, 91.7, 107.5, 122.1, 126.9, 128.3, 128.4, 128.5, 128.6, 128.7, 130.0, 135.9, 136.0, 141.8, 149.7, 151.2, 152.9. Anal. Calcd for $C_{31}H_{37}N_5O_6Si\cdot H_2O$: 59.88 (C); 6.32 (H); 11.26 (N). Found: 59.81 (C); 6.09 (H); 11.01 (N).

4.5. 6-*N*-Benzyloxycarbonyl-2′,3′-*O*-benzylideneadenosine (10)

TBAF (1 M in THF, 10 mL) was added to a solution of **9** (1.5 g, 2.5) in dry THF (10 mL), the reaction mixture was stirred overnight at rt under N_2 atmosphere. TLC (MeOH/DCM, 1:10, v/v), showed traces of starting material and additional TBAF solution (1 M in THF, 3 mL) was added and the reaction mixture was stirred for additional 5 h at rt under N_2 atmosphere. The reaction was quenched by the addition of Dowex x 50w (20 mL mix of resin in pyridine) and the reaction mixture stirred gently for 20 min. The Dowex was filtered off by suction, washed with a pyridine/methanol/water solution (3:1:1, 3×20 mL) and the solvents were removed under reduced pressure and co-distilled with toluene. The crude product was purified by automated flash chromatography (stepwise gradient, 2–20% v/v, MeOH in DCM), yielding compound **10** as a white foam (2.1 g, 95%). R_f=0.5 (MeOH/DCM, 1:10, v/v). ^{1}H NMR ($CDCl_3$) δ 3.80–3.92 (2H, m), 4.63 (1H, s), 5.13–5.24 (4H, m), 5.92 (1H, d, *J* 4.4 Hz), 6.00 (1H, s), 7.24–7.42 (10H, m), 7.95 (1H, s), 8.68 (1H, s), 9.63 (1H, s). ^{13}C NMR ($CDCl_3$) δ 62.9, 67.7, 83.3, 83.4, 86.0, 93.1, 107.5, 123.0, 126.5, 128.5, 128.6, 128.6, 129.9, 135.2, 135.7, 142.7, 150.0, 150.2, 151.1, 152.4. Anal. Calcd for $C_{25}H_{23}N_5O_6\cdot\frac{1}{2}H_2O$: 60.27 (C); 5.06 (H); 14.05 (N). Found: 60.39 (C); 4.98 (H); 14.11 (N).

4.6. 6-*N*-Benzyloxycarbonyl-2′,3′-*O*-benzylidene-5′-sulfamoyladenosine (11)

Compound **10** (1.7 g, 3.50 mmol) was dissolved in dry DCM (10 mL) at 0 °C. DMAP (1.0 g, 8.2 mmol) was added and the reaction mixture was stirred for 20 min. Sulfamoyl chloride (0.9 g, 7.8 mmol) suspended in dry DCM (2 mL) was added dropwise to the reaction mixture at 0 °C, the pH was carefully monitored during the addition and was kept at pH 7 by adding small portions of DMAP when pH<7. The mixture was stirred overnight at rt under N_2 atmosphere. The reaction was quenched by the addition of H_2O (50 mL). EtOAc (100 mL) was added, the layers were separated and the aqueous phase was extracted with EtOAc (3×100 mL). The pooled organic layers were washed with H_2O (100 mL) and brine (100 mL), dried over $MgSO_4$ and filtered before the solvent was removed under reduced pressure. The crude product was purified using automated flash chromatography (stepwise gradient, 2–20% v/v, MeOH in DCM), yielding compound **11** as a white foam (1.9 g, 96%). R_f=0.4 (MeOH/DCM, 1:10, v/v). ^{1}H

NMR ($CDCl_3$) δ 4.20–4.38 (2H, m), 4.64 (1H, q, *J* 6.2 and 2.2 Hz), 5.07–5.12 (3H, m), 5.42 (1H, dd, *J* 6.6 and 1.8 Hz), 5.91 (1H, s), 6.18 (3H, br s), 7.21–7.50 (10H, m), 8.05 (1H, s), 8.62 (1H, s), 9.44 (1H, s). ^{13}C NMR ($CDCl_3$) δ 68.0, 69.3, 82.3, 84.6, 84.8, 90.7, 107.9, 122.2, 126.9, 128.6, 128.8, 130.3, 135.4, 142.6, 149.5, 150.8, 151.6, 152.8. Anal. Calcd for $C_{25}H_{24}N_6O_8S\cdot H_2O$: 51.19 (C); 4.47 (H); 14.33 (N). Found: 51.08 (C); 4.29 (H); 14.11 (N).

4.7. General procedure for the synthesis of 6-*N*-benzyloxycarbonyl-2′,3′-*O*-benzylidene-5′-*O*-[*N*-(aminoacyl)-sulfamoyl]adenosines (12a–h)

The protected amino acid (3 equiv) was dissolved in dry DCM and dry DMF if necessary (6 mL). PL-DCC (1.5 mmol loading, 4 equiv) was added, the microvial was capped and stirred gently at rt under N_2 atmosphere for 1 h. Compound **11** (1 equiv) and DMAP (3 equiv) were dissolved in dry DCM (1 mL) and added to the reaction mixture, which was stirred gently at rt under N_2 atmosphere for 24 h. The resin was filtered off and washed with dry DCM (3×10 mL) and MeOH (3×10 mL). The solvents were removed under reduced pressure. The crude products were purified using automated flash chromatography (stepwise gradient, 2–20% v/v, MeOH in DCM) followed by preparative HPLC (gradient from 27.5% MeCN in 50 mM phosphate buffer to 45% ACN and 45% MeOH in 50 mM, phosphate buffer, pH 7.0 and a flow of 15 mL/min).

*4.7.1. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz-L-valinyl)sulfamoyl]adenosine (**12a**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-Cbz-L-valine (0.27 g, 1.06 mmol) yielding **12a** (0.24 g, 85%) as a beige foam. R_f=0.4 (MeOH/DCM, 1:10, v/v). ^{1}H NMR (CD_3CN) δ 0.76 (6H, d, *J* 6.82 Hz), 0.86 (6H, d, *J* 6.75 Hz), 1.98–2.12 (2H, m), 3.80–3.90 (2H, m), 4.18–4.24 (4H, m), 4.60 (1H, q, *J* 3.82 and 7.83 Hz), 4.68–4.72 (1H, m), 4.92–5.12 (5H, m), 5.15–5.25 (5H, m), 5.36 (1H, s), 5.38 (1H, s), 5.94 (1H, s), 6.16 (1H, s), 6.35 (1H, d, *J* 2.29 Hz), 6.37 (1H, d, *J* 2.29 Hz), 7.21–7.56 (35H, m), 8.53 (1H, s), 8.58 (2H, s), 8.60 (1H, s). ^{13}C NMR (CD_3CN) δ 18.1, 20.3, 32.5, 63.6, 63.7, 67.1, 68.3, 69.2, 82.4, 84.2, 84.9, 85.4, 86.1, 86.4, 91.0, 91.9, 105.1, 108.3, 123.4, 128.0, 128.1, 128.7, 128.7, 128.9, 129.3, 129.4, 129.5, 129.6, 129.7, 131.0, 131.1, 137.2, 137.3, 137.3, 138.4, 144.0, 150.5, 150.5, 152.4, 152.5, 153.1, 153.3, 157.9, 179.7. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{38}H_{39}N_7O_{11}S$: 802.2501. Found: 802.2500.

*4.7.2. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz-L-lysinyl(ε-Cbz))sulfamoyl]adenosine (**12b**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-Cbz-L-lysine(ε-Cbz) (0.44 g, 1.06 mmol) yielding **12b** (0.18 g, 53%) as a white foam. R_f=0.4 (MeOH/DCM, 1:10, v/v). ^{1}H NMR (CD_3CN) δ 1.29–1.44 (8H, m), 1.50–1.61 (2H, m), 1.68–1.79 (2H, m), 2.96–3.08 (4H, m), 3.85–3.91 (2H, m), 4.12–4.20 (4H, m), 4.62–4.68 (1H, m), 4.71–4.77 (1H, m), 4.98–5.08 (7H, m), 5.11–5.13 (1H, m), 5.17–5.25 (5H, m), 5.34–5.42 (3H, m), 5.72 (1H, br s), 5.93–5.98 (3H, m), 6.20 (1H, s), 6.37 (1H, d, *J* 2.29 Hz), 6.38–6.40 (2H, m), 7.24–7.61 (40H, m), 8.59 (1H, s), 8.61 (1H, s), 8.63 (1H, s), 8.70 (1H, s). ^{13}C NMR (DMSO-d_6) δ 22.9, 29.0, 57.0, 65.1, 65.1, 66.3, 37.1, 80.8, 83.3, 83.8, 88.1, 89.6, 103.1, 106.5, 123.3, 126.9, 127.0, 127.5, 127.6, 127.7, 127.9, 128.0, 128.3, 128.4, 128.4, 128.5, 129.7, 129.9, 134.0, 136.1, 136.3, 137.2, 137.3, 149.7, 151.5, 152.0, 155.8, 155.9, 155.9, 156.1, 176.9. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{47}H_{48}N_8O_{13}S$: 965.3134. Found: 965.3133.

*4.7.3. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(2,4-di(benzyloxycarbonylamino)-L-butyryl)sulfamoyl]adenosine (**12c**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*,*N*′-Cbz-L-2,4-diaminobutanoic acid (0.41 g, 1.06 mmol) yielding **12c** (0.16 g, 49%) as white foam. ^{1}H NMR (CD_3CN) δ 1.61–1.74 (2H, m), 1.90–1.98 (2H, m), 3.02–3.17 (4H, m), 3.93–4.03 (2H, m), 4.15–4.21 (4H, m), 4.54–4.59 (1H, m), 4.64–4.69 (1H, m), 4.90–5.20 (14H, m), 5.31 (2H, s), 5.89 (1H, s), 6.13 (1H, s), 6.30–6.36 (2H, m), 7.17–7.54 (44H, m), 8.55 (1H, s), 8.57 (1H, s), 8.58 (1H, s), 8.62 (1H, s). ^{13}C NMR (CD_3CN) δ 25.5, 31.5, 34.3, 36.8, 38.5, 38.5, 43.6, 54.2, 56.2, 66.9, 67.1, 67.1, 67.2, 67.4, 68.2, 68.7, 69.1, 69.2, 82.3, 84.1, 84.9, 85.4, 86.1, 86.3, 90.9, 91.9, 104.9, 108.1, 123.3, 127.9, 128.0, 128.5, 128.7, 128.8, 128.8, 128.9, 128.9, 129.0, 129.1, 129.2, 129.3, 129.3, 123.4, 129.5, 129.5, 129.5, 129.6, 129.6, 129.7, 130.8, 131.0, 136.80, 137.0, 137.1, 137.2, 138.0, 138.1, 138.3, 143.9, 150.3, 150.3, 152.3, 152.4, 153.0, 153.2, 153.2, 157.5, 157.6, 163.7, 173.2, 179.5. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{45}H_{44}N_8O_{13}S$: 937.2821. Found: 937.2828.

*4.7.4. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz-L-ornithinyl(δ-Cbz))-sulfamoyl]adenosine (**12d**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-Cbz-L-ornithine(δ-Cbz) (0.42 g, 1.06 mmol) yielding **12d** (0.19 g, 73%) as white foam. ^{1}H NMR (CD_3CN) δ 1.42–2.22 (8H, m), 2.89–2.99 (4H, m), 3.86–3.98 (2H, m), 4.08–4.16 (4H, m), 4.62–4.73 (2H, m), 4.91–5.14 (14H, m), 5.24–5.31 (2H, m), 5.80 (2H, br s), 5.89 (1H, s), 6.03 (1H, s), 6.30 (2H, s), 7.20–7.54 (40H, m), 8.55 (2H, s), 8.67 (2H, s), 9.45 (1H, br s). ^{13}C NMR (CD_3CN) δ 26.8, 31.1, 41.2, 58.0, 66.8, 67.0, 68.3, 69.1, 82.2, 84.9, 85.4, 86.1, 86.3, 90.8, 90.8, 91.8, 104.9, 104.9 108.1, 123.3, 127.9, 128.0, 128.7, 128.8, 128.8, 129.1 129.2, 129.4, 129.4, 123.5, 129.5, 130.8, 131.0, 137.0, 137.1, 138.2, 138.4, 144.0, 150.3, 150.3, 152.3, 152.3, 153.1, 153.2, 157.4, 157.6, 157.7, 180.0. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{46}H_{48}N_8O_{13}S$: 951.2978. Found: 951.2972.

*4.7.5. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz)-L-homoserinyl(Bn)sulfamoyl]adenosine (**12e**).* Following the general procedure compound **11** (0.2 g, 0.35 mmol) was reacted with *N*-Cbz-L-homoserine(OBn) (0.36 g, 1.06 mmol) yielding **12e** (0.16 g, 51%) as white foam. ^{1}H NMR (CD_3CN) δ 1.71–1.86 (2H, m), 1.94–2.08 (2H, m), 3.37–3.49 (4H, m), 4.06–4.20 (4H, m), 4.23–4.39 (2H, m), 4.54–4.61 (1H, m), 4.64–4.71 (1H, m), 4.88–5.19 (10H, m), 5.22–5.32 (2H, m), 5.90 (1H, s), 6.14 (1H, s), 6.37 (2H, s), 7.18–7.54 (44H, m), 8.50–8.58 (3H,m), 8.66 (1H, s). ^{13}C NMR (CD_3CN) δ 31.4, 33.6, 36.7, 56.2, 56.3, 68.1, 68.2, 73.4, 82.2, 84.0, 84.9, 85.4, 86.1, 86.4, 90.8, 91.8, 104.9, 108.0, 123.3, 127.7, 127.9, 128.0, 128.3, 128.6, 128.7, 129.0, 129.1, 129.2, 129.4, 129.5, 129.5, 129.8, 130.8, 131.0, 137.1, 137.2, 138.2, 139.8, 144.0, 150.3, 152.4, 152.5, 153.0, 153.1, 157.4, 163.6, 180.0. HRMS *m*/*z* $[M+H]^+$ calculated for $C_{44}H_{43}N_7O_{12}S$: 894.2763. Found: 894.2750.

*4.7.6. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz)-L-serinyl(P(O)(OBn)$_2$)sulfamoyl]adenosine (**12f**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-Cbz-L-serine phosphate$(OBn)_2$ (0.05 g, 0.11 mmol) yielding **12f** (0.02 g, 61%) as white foam. ^{1}H NMR (CD_3CN) δ 4.12–4.18 (4H, m), 4.26–4.67 (8H, m), 4.92–5.02 (12H, m), 5.10–5.17 (2H, m), 5.26–5.30 (2H, m), 5.86 (1H, s), 6.13 (1H, s), 6.24–6.30 (3H, m), 7.21–7.55 (52H, m), 8.57 (1H, s), 8.58 (1H, s), 8.61 (1H, s), 8.61 (1H, s). ^{13}C NMR (CD_3CN) δ 58.2, 58.2, 67.1, 68.1, 69.0, 69.3, 69.4, 70.2, 70.2, 70.4, 81.5, 82.3, 84.1, 85.1, 85.6, 86.1, 86.4, 91.1, 92.1, 104.9, 108.0, 123.4, 127.9, 128.0, 128.7, 128.8, 129.0, 129.1, 129.2, 129.3, 129.4, 129.5, 129.5, 129.7, 130.8, 131.0, 137.0, 137.1, 137.2, 137.5, 138.1, 143.8, 145.4, 150.3, 150.4, 152.4, 152.45, 152.5, 152.9, 153.0, 153.1, 156.6, 157.1, 157.1, 174.1, 175.3, 175.4. HRMS *m*/*z* $[M+H+K]^+$ calculated for $C_{50}H_{48}N_7O_{15}PS$: 1088.2298. Found: 1088.2229.

*4.7.7. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz-L-tyrosyl(OBn))sulfamoyl]adenosine (**12g**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-

Cbz-L-tyrosine(OBn) (0.43 g, 1.06 mmol) yielding **12g** (0.20 g, 60%) as white foam. ^{1}H NMR (CD_3CN) δ 3.01–3.06 (2H, m), 4.12–4.18 (8H, m), 4.57–4.63 (1H, m), 4.68–4.75 (1H, m), 4.87–5.45 (18H, m), 6.17 (1H, s), 6.33 (1H, s), 6.34–6.50 (2H, m), 6.76–7.07 (8H, m), 7.21–7.57 (44H, m), 8.55 (2H, s), 8.61 (2H, s). ^{13}C NMR (CD_3CN) δ 37.4, 38.5, 56.7, 59.6, 67.8, 67.1, 67.7, 68.2, 69.0, 69.1, 70.5, 70.6, 82.2, 84.0, 84.9, 85.3, 86.0, 86.3, 90.9, 91.9, 104.9, 108.0, 115.3, 115.7, 123.4, 127.9, 128.0, 128.5, 128.6, 128.7, 128.8, 128.9, 128.9, 129.1, 129.2, 129.2, 129.3, 129.4, 129.5, 130.0, 130.8, 131.0, 131.4, 131.5, 136.9, 137.0, 137.1, 138.1, 138.2, 137.5, 143.8, 150.4, 150.5, 152.3, 152.4, 153.1, 157.0, 157.1, 158.3, 158.7, 172.8, 179.3, 179.3. HRMS m/z $[M+H]^+$ calculated for $C_{49}H_{45}N_7O_{12}S$: 956.2920. Found: 956.2921.

*4.7.8. 6-N-Benzyloxycarbonyl-2′,3′-O-benzylidene-5′-O-[N-(N-Cbz-L-tryptophanyl)sulfamoyl]adenosine (**12h**).* Following the general procedure compound **11** (0.20 g, 0.35 mmol) was reacted with *N*-Cbz-L-tryptophan (0.54 g, 1.58 mmol) yielding **12h** (0.13 g, 53%) as white foam. ^{1}H NMR (CD_3CN) δ 2.90–3.08 (2H, m), 3.16–3.21 (2H, m), 4.10–4.17 (4H, m), 4.30–4.32 (2H, m), 4.51–4.60 (1H, m), 4.61–4.68 (1H, m), 4.82–5.03 (5H, m), 5.05–5.38 (7H, m), 5.83 (1H, s), 6.12 (1H, s), 6.31 (2H, br s), 6.88–7.53 (44H, m), 8.49 (1H, s), 8.56–8.66 (3H, m). ^{13}C NMR (CD_3CN) δ 29.3, 59.1, 66.9, 68.3, 69.1, 69.1, 82.3, 84.1, 84.9, 85.4, 86.0, 86.3, 90.9, 91.0, 92.0, 105.1, 108.1, 111.8, 111.9, 112.3, 112.4, 119.6, 119.8, 122.3, 123.3, 124.5, 124.7, 128.0, 128.1, 128.6, 128.8, 129.0, 129.2, 129.4, 129.5, 129.6, 130.9, 131.0, 137.2, 137.2, 137.4, 138.3, 143.9, 150.3, 150.4, 152.4 152.5, 153.0, 153.0, 153.1, 153.2, 157.3, 179.8. HRMS m/z $[M+H]^+$ calculated for $C_{44}H_{40}N_8O_{11}S$: 889.2610. Found: 889.2618.

4.8. General procedure for the synthesis of 5′-*O*-(*N*-aminoacyl)sulfamoyladenosines (13a–h)

6-*N*-Benzyloxycarbonyl-2′,3′-*O*-benzylidene-5′-*O*-[(*N*-aminoacyl)sulfamoyl]adenosines (**12a**–**h**) (0.03 mmol) were dissolved in water/2-propanol (5:95 v/v, 6 mL) and hydrogenation was carried out in an continuous-flow hydrogenation reactor (H-cube®) (flow rate:1 mL/min; pressure: 50 bar; temperature: 70 °C; catalyst: 10% Pd/C CatCart, 30×4 mm; time: 120 min). The solvent was removed under reduced pressure and the compounds were purified using reversed phase preparative HPLC using Method One (mobile phase; gradient 5–95% MeCN in water, 0.2% NH_3; column: Waters Xbridge Prep C18 5 μm OBD™ 19×150 mm) or Method Two (mobile phase; gradient 0–20% MeCN in water, 0.01% TFA; Column: Waters Xbridge Prep C18 5 μm 10×150 mm).

*4.8.1. 5′-O-(N-Valinyl)sulfamoyladenosine (**13a**).* Following the general procedure compound **12a** (30 mg, 0.03 mmol) was hydrogenated to yield **13a** (8 mg, 58%) as a white foam (HPLC Method Two). $[\alpha]_D^{20}$ −0.10 (*c* 0.42, DMSO). ν_{max} (DMSO) 3440, 3250, 3070, 2250, 2130, 1620, 1310, 1220 cm^{-1}. ^{1}H NMR (D_2O) δ 0.7–0.11 (6H, m), 2.18–2.23 (1H, m), 3.75 (1H, d, *J* 6.2 Hz), 4.46–4.21 (3H, m), 4.51 (1H, d, *J* 4.5 Hz), 4.72 (1H, t, *J* 5.3 Hz), 6.15 (1H, d, *J* 5.3 Hz), 8.29 (1H, s), 8.39 (1H, s). δ_C (100 MHz; D_2O): 18.31, 18.35, 31.11, 58.13, 62.4, 69.4, 73.02, 82.11, 96.81, 119.01, 140.25, 149.27, 152.40, 156.91, 174.80. HRMS m/z $[M+H]^+$ calculated for $C_{15}H_{23}N_7O_7S$: 446.1458. Found: 446.1461.

*4.8.2. 5′-O-(N-Lysinyl)sulfamoyladenosine (**13b**).* Following the general procedure compound **12b** (30 mg, 0.03 mmol) was hydrogenated to yield **13b** (5 mg, 36%) as a white foam (HPLC Method Two). $[\alpha]_D^{20}$ −0.04 (*c* 0.42, DMSO). ν_{max} (DMSO) 3440, 3270, 3070, 2970, 2870, 2250, 2130, 1770 cm^{-1}. ^{1}H NMR (D_2O) δ 1.19–1.26 (2H, m), 1.47–1.54 (2H, m), 1.78–182 (2H, m), 2.63–2.70 (2H, m), 3.59 (1H, t, *J* 6.2 Hz), 4.47–4.21 (3H, m), 4.52 (1H, t, *J* 4.5 Hz), 4.72 (1H, t, *J* 5.3 Hz), 6.17 (1H, d, *J* 5.3 Hz), 8.31 (1H, s), 8.38 (1H, s). δ_C (100 MHz; D_2O) 22.31, 28.71, 33.42, 42.09, 53.21, 63.11, 70.84, 72.87, 81.89, 97.01, 119.40, 140.21, 149.87, 151.98, 155.93, 175.85. HRMS m/z $[M+H]^+$ calculated for $C_{16}H_{26}N_8O_7S$: 475.1723. Found: 475.1724.

*4.8.3. 5′-O-(N-2,4-Diaminobuturyl)sulfamoyladenosine (**13c**).* Following the general procedure compound **12c** (30 mg, 0.03 mmol) was hydrogenated to yield **13c** (11 mg, 78%) as a white foam (HPLC Method One). $[\alpha]_D^{20}$ −0.25 (*c* 0.83, DMSO). ν_{max} (DMSO) 3450, 3270, 3070, 2250, 2130, 1660 cm^{-1}. ^{1}H NMR (D_2O) δ 1.98–2.05 (2H, m), 2.68–2.73 (2H, m), 3.75 (1H, t, *J* 6.2 Hz), 4.45–4.19 (3H, m), 4.51 (1H, t, *J* 4.5 Hz), 4.71 (1H, t, *J* 5.3 Hz), 6.16 (1H, d, *J* 5.3 Hz), 8.26 (1H, s), 8.35 (1H, s). δ_C (100 MHz; D_2O) 33.25, 37.15, 50.61, 62.89, 69.88, 72.79, 82.19, 96.86, 141.92, 149.29, 152.41, 156.34, 174.24. HRMS m/z $[M+H]^+$ calculated for $C_{14}H_{20}N_8O_7S$: 445.1254. Found: 445.1257.

*4.8.4. 5′-O-(N-Ornithinyl)sulfamoyladenosine (**13d**).* Following the general procedure compound **12d** (30 mg, 0.03) was hydrogenated to yield **13d** (3 mg, 21%) as a white foam (HPLC Method One) $[\alpha]_D^{20}$ −0.34 (*c* 0.83, DMSO). ν_{max} (DMSO) 3470, 3430, 2970, 2920, 2870, 2250, 2130, 1660, 1450 cm^{-1}. ^{1}H NMR (D_2O) δ 1.46–1.52 (2H, m), 1.77–1.82 (2H, m), 2.70–2.75 (2H, m), 3.61 (1H, t, *J* 6.2 Hz), 4.48–4.22 (3H, m), 4.54 (1H, t, *J* 4.5 Hz), 4.70 (1H, t, *J* 5.3 Hz), 6.15 (1H, d, *J* 5.3 Hz), 8.31 (1H, s), 8.36 (1H, s). δ_C (100 MHz; D_2O) 24.11, 25.03, 41.34, 53.16, 62.54, 72.32, 82.06, 95.11, 150.10, 152.44, 156.21, 175.02. HRMS m/z $[M+H]^+$ calculated for $C_{15}H_{24}N_8O_7S$: 461.1567. Found: 461.1569.

*4.8.5. 5′-O-(N-Homoserinyl)sulfamoyladenosine (**13e**).* Following the general procedure compound **12e** (20 mg, 0.02 mmol) was hydrogenated to yield **13e** (3 mg, 39%) as a white foam (HPLC Method One). $[\alpha]_D^{20}$ −0.28 (*c* 0.83, DMSO). ν_{max} (DMSO) 3450, 3270, 3070, 2920, 2250, 2130, 2000, 1620 cm^{-1}. ^{1}H NMR (D_2O) δ 1.96–2.01 (2H, m), 3.55–3.63 (2H, m), 3.64 (1H, t, *J* 6.2 Hz), 4.47–4.21 (3H, m), 4.52 (1H, t, *J* 4.5 Hz), 4.73 (1H, t, *J* 5.3 Hz), 6.16 (1H, d, *J* 5.3 Hz), 8.31 (1H, s), 8.42 (1H, s). δ_C (100 MHz; D_2O) 26.21, 29.93, 53.11, 62.12, 63.90, 82.19, 95.78, 119.34, 140.13, 149.39, 152.32, 156.02, 174.60. HRMS m/z $[M+H]^+$ calculated for $C_{14}H_{21}N_7O_8S$: 448.1251. Found: 448.1254.

*4.8.6. 5′-O-(N-Serinylphosphate)sulfamoyladenosine (**13f**).* Following the general procedure compound **12f** (30 mg, 0.03 mmol) was hydrogenated to yield **13f** (12 mg, 77%) as a white foam (HPLC Method One). $[\alpha]_D^{20}$ −0.07 (*c* 0.42, DMSO). ν_{max} (DMSO) 3440, 3250, 3070, 2920, 2250, 2120, 2000, 1620 cm^{-1}. ^{1}H NMR (D_2O) δ 3.66 (1H, t, *J* 6.1 Hz), 4.29–4.23 (1H, m), 4.47–4.21 (3H, m), 4.51–4.47 (1H, m), 4.67–4.62 (1H, m), 4.75–4.69 (1H, m), 6.11 (1H, d, *J* 5.2 Hz), 8.35 (1H, s), 8.49 (1H, s). δ_C (100 MHz; D_2O) 52.71, 58.73, 62.10, 70.10, 72.83, 82.14, 95.88, 119.41, 149.52, 152.42, 156.07, 175.33. HRMS m/z $[M+H]^+$ calculated for $C_{13}H_{18}N_7O_{11}PS+2Na^+$: 558.3485. Found, 558.3487.

*4.8.7. 5′-O-(N-Tyrosyl)sulfamoyladenosine (**13g**).* Following the general procedure compound **12g** (40 mg, 0.04 mmol) was hydrogenated to yield **13g** (13 mg, 66%) as a white foam (HPLC Method Two). $[\alpha]_D^{20}$ −0.08 (*c* 0.42, DMSO). ν_{max} (DMSO) 3440, 3250, 3070, 2920, 2250, 2130, 2000, 1620 cm^{-1}. LCMS ESI$^+$=510.5. NMR data were in agreement with published data.[11]

*4.8.8. 5′-O-(N-Tryptophanyl)sulfamoyladenosine (**13h**).* Following the general procedure compound **12h** (30 mg, 0.03 mmol) was hydrogenated to yield **13h** (10 mg, 60%) as a white foam (HPLC Method Two). $[\alpha]_D^{20}$ −0.05 (*c* 0.42, DMSO). ν_{max} (DMSO) 3510, 3470,

3010, 2920, 2250, 2130, 2000, 1670 cm^{-1}. LCMS ESI^{+}=534.6 NMR data were in agreement with published data.[35]

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