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Base substituted 5′-*O*-(*N*-isoleucyl)sulfamoyl nucleoside analogues as potential antibacterial agents

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ABSTRACT

Aminoacyl-sulfamoyl adenosines are well-known nanomolar inhibitors of the corresponding prokaryotic and eukaryotic tRNA synthetases in vitro. Inspired by the aryl-tetrazole containing compounds of Cubist Pharmaceuticals and the modified base as found in the natural antibiotic albomycin, the selectivity issue of the sulfamoylated adenosines prompted us to investigate the pharmacophoric importance of the adenine base. We therefore synthesized and evaluated several isoleucyl-sulfamoyl nucleoside analogues with either uracil, cytosine, hypoxanthine, guanine, 1,3-dideaza-adenine (benzimidazole) or 4-nitro-benzimidazole as the heterocyclic base. Based on the structure and antibacterial activity of microcin C, we also prepared their hexapeptidyl conjugates in an effort to improve their uptake potential. We further compared their antibacterial activity with the parent isoleucyl-sulfamoyl adenosine (Ile-SA), both in in vitro and in cellular assays. Surprisingly, the strongest in vitro inhibition was found for the uracil containing analogue **16f**. Unfortunately, only very weak growth inhibitory properties were found as of low uptake. The results are discussed in the light of previous literature findings.

1. Introduction

The problem of antibiotic resistance among pathogenic bacteria is as old as antibiotics itself.[1] Due to the emergence of multidrug resistant pathogens, there is a continuous quest for new cellular targets which are vital for survival of the pathogen or for new antimicrobial agents interfering with known targets.[2] In recent years, aminoacyl-tRNA synthetases (aaRSs) have emerged as attractive and clinically validated targets for antimicrobial drug development as exemplified by the clinical use of mupirocin [isoleucine-tRNA synthetase (IleRS) inhibitor].[3] More recently different borate containing small molecules have progressed into clinical trials, and are targeting the editing site of aaRSs, further underpinning the idea of aaRSs being an excellent target for antibiotic development.[4–8] These enzymes are at the heart of translation and responsible for ligation of the correct amino acid to their cognate tRNA.[5,9] Aminoacylation of tRNA occurs in a two-step process. The first step involves formation of aminoacyl-adenylate (aa-AMP) followed by transfer of the aminoacyl moiety to the 3′-terminal adenosine of the respective tRNA. These aminoacyl-tRNAs are further used in translation and are pivotal in determining fidelity of protein synthesis. Because of their crucial role in protein synthesis, inhibition of these enzymes proves detrimental to the cell.[10] Although, aaRSs are essential in all living organisms, selective inhibition of bacterial aaRS over their human orthologs is a big hurdle for discovering novel aaRS inhibitors as potential antibiotics.

The early efforts in the search for aaRS inhibitors yielded diverse sets of chemical structures which are either a substrate mimic or mimic of the reaction intermediate (aa-AMP). The largest group of aaRS inhibitors are analogues of the aa-AMP in which the labile acyl-phosphate linkage is replaced with relatively stable aminoacyl-sulfamates,[11–15] aminoalkyl phosphates,[11,12,16] esters,[17–19] amides,[17] hydroxamates,[17–19] sulfamides,[11,12] *N*-alkoxysulfamides or *N*-hydroxysulfamides.[20] Among them, aminoacyl-sulfamate derivatives yielded the more potent inhibitors. Isoleucyl sulfamoyl adenosine (IleSA, **1a**, Fig. 1) is given as an example for this class of inhibitors. Unfortunately, these analogues lack selectivity for bacterial aaRSs as compared to their human aaRSs due to structural similarity with aa-AMP. In addition, the lack of cell penetration further limits their clinical use.

Several modifications have been attempted in order to improve the selectivity of these aminoacyl-sulfamoyl adenosine analogues. In 1998, Cubist Pharmaceuticals reported the synthesis and evaluation of a new class of aaRS inhibitors where the adenine base

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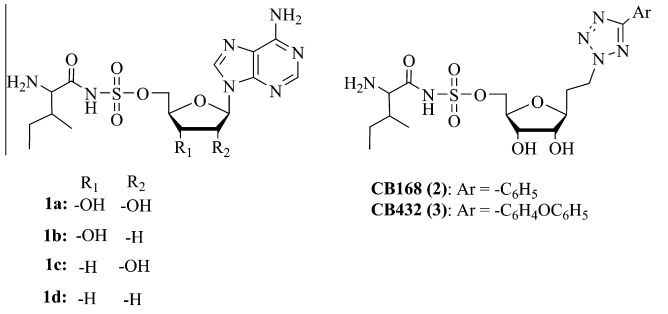


Figure 1. Isoleucyl-sulfamoyl adenosine (**1a**), its deoxygenated variants (**1b–d**) and the aryl-tetrazole containing analogues (**2–3**) from Cubist Pharmaceuticals as IleRS inhibitors.

is replaced with an aryl-tetrazole moiety and linked to the sugar part through a two carbon chain. The most important compounds of this series (**2** and **3**) are likewise depicted in Figure 1. These compounds exhibit excellent potency (IC_{50} values in the nanomolar range) with improved selectivity (up to 3000-fold) over human IleRS. However, further development was halted due to their lack of in vivo activity and high serum albumin binding.[3,21] Further replacement of ribofuranose of **1a** with 2′- or 3′-deoxy or 2′,3′-dideoxy sugars (**1b–d** Fig. 1) yielded less potent aaRS inhibitors. The 2′-deoxy, 3′-deoxy and 2′,3′-dideoxy analogues displayed 1300-fold, 2600-fold and 4-fold reduction in enzyme inhibition, respectively, as compared to IleSA (**1a**).[22]

An example of the use of a modified base to substitute for adenine can be found in nature as well, wherein albomycin is produced by *Streptomyces* species as a mixture of the closely related substances (**4a–c**, Fig. 2) being a natural Trojan-Horse antibiotic.[23] Especially albomycin $\delta 2$ (**4b**) is highly effective against many Gram-negative and some Gram-positive pathogens (with a MIC of 10 ng/mL against *Streptococcus pneumonia*).[24] Albomycin is a so called siderophore–drug conjugate (SDC) and consists of the antibiotic moiety linked to an iron carrier part (ornithine-based trihydroxamate) called the siderophore and promoting its uptake. A modified pyrimidine is attached at the 1′-position of a thioribofuranosyl moiety. Once internalized via an ATP dependent iron channel, the ferric ion is reduced to ferrous cation by ferric reductase and the siderophore part is metabolized by non-specific peptidases. This in turn releases the active moiety which resembles seryl-adenosine monophosphate (SerAMP) and inhibits seryl-tRNA synthetase (SerRS), but shows considerable deviation of the standard adenine base moiety.[25]

Microcin C (McC) (**5a**, Fig. 2) on the other hand is a natural antibiotic produced by *Escherichia coli* and consists of a formylated heptapeptide of which the terminal aspartic acid is attached to a modified AMP via a phosphoramidate linkage to its α-carboxylic acid moiety.[26,27] As of its peptidic signature, it is actively taken up by *E. coli* through the YejABEF transporter.[26] Once internalized, it is processed by a peptide deformylase and one of the three aminopeptidases, pepA, pepB or pepN to release a non-hydrolyzable analogue of aspartyl adenylated (**5b**, Fig. 2) which selectively inhibits aspartyl-tRNA synthetase (AspRS).[28] In view of their prodrug like properties, both albomycin and McC therefore are called Trojan-Horse antibiotics.

The high selectivity of aryl-tetrazole containing sulfamates[3,21] and the excellent efficacy of albomycin against *Streptococcus pneumonia*[24] both deviating from the adenine base structure while yet acting as aaAMP analogues, prompted us to investigate the pharmacophoric importance of the adenine base for the well-known aminoacyl sulfamoyl adenosine (aaSA) inhibitors. We therefore intended to evaluate a series of aaSA analogues with different natural or unnatural heterocyclic bases substituting for the adenine ring. To our knowledge, such substitution of the adenine base has not been attempted so far. Towards this end, we designed, synthesized, and evaluated several isoleucyl-sulfamoyl nucleoside analogues with either uracil (U), cytosine (C), hypoxantine (I), guanine (G), 1,3-dideaza-adenine [4-amino-benzimidazole (4-ABI)] or 4-nitro-benzimidazole (4-NBI) as the heterocyclic base. We further compared their antibacterial activity with the parent Ile-SA, both in vitro and in cellular assays.

2. Chemistry

The corresponding series of isoleucyl sulfamate analogues were prepared combining different literature procedures. The required nitrobenzimidazole heterocycle was synthesized starting from *o*-phenylene diamine via its selenium complex[29] followed by nitration,[30] reduction,[31] and cyclization[32] using triethyl orthoformate yielding 4(7)-nitrobenzimidazole (Scheme 1).

The heterocyclic base was further reacted with tetra-*O*-acetyl ribofuranose using stannic chloride[32] followed by deprotection of the acetyl moieties using methanolic ammonia yielding the 4-nitro benzimidazole nucleoside analogue **12a**. All sulfamoylated nucleoside analogues (natural and unnatural ones) were further synthesized following literature procedures which involved persilylation of the nucleoside followed by selective removal of the 5′-TBDMS protection and reaction of the liberated 5′-hydroxyl group with in situ formed sulfamoyl chloride.[33] Finally, the isoleucyl sulfamate analogues were obtained by condensation of the active ester of Boc-Ile (in form of Boc-Ile-OSu) with the respective sulfamoylated analogues (**15a–f**) using DBU as a base in DMF. The Boc group was deprotected by acidolysis using TFA/water and TBDMS groups were cleaved by $Et_3N{\cdot}3HF$ affording the desired isoleucyl sulfamate analogues (**16a–f**). Finally, the 4-nitro moiety in **16a** was reduced by hydrogenation using Pd/C under H_2 atmosphere to yield **16g** (Scheme 2). The hexapeptide uptake signal was further coupled to the analogues **16a–g** to afford derivatives **17a–g** following a previous protocol[34] (Scheme 3).

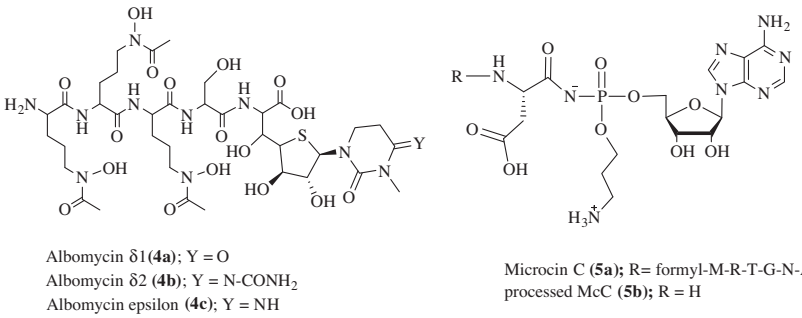
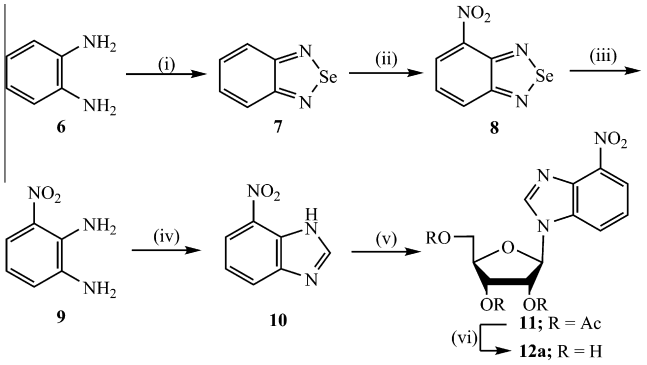


Figure 2. Albomycin congeners (**4a–c**) and microcin C (**5a**) as examples of Trojan-Horse antibiotics.



Scheme 1. Synthesis of 1-(β-D-ribofuranosyl)-4-nitrobenzimidazole Reagents and conditions: (i) ethanol, selenium dioxide, reflux 10 min, 99%; (ii) concd H_2SO_4 and concd HNO_3 from 0° to rt in 30 min, 100%; (iii) concd HCl, aq HI (57%), 3 h, 54%; (iv) triethyl orthoformate, reflux, 4 h then formic acid relux 3 h, 87%; (v) tetra-*O*-acetyl ribofuranose, $SnCl_4$ (1 M solution in DCM), dry ACN, 25 °C, 16 h, 80%; (vi) 7 N ammonia in methanol, rt, overnight, 90%.

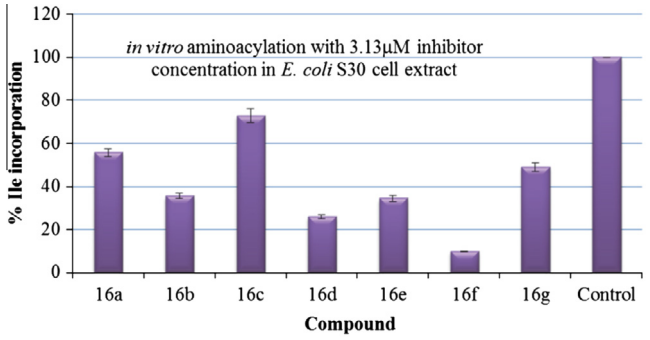


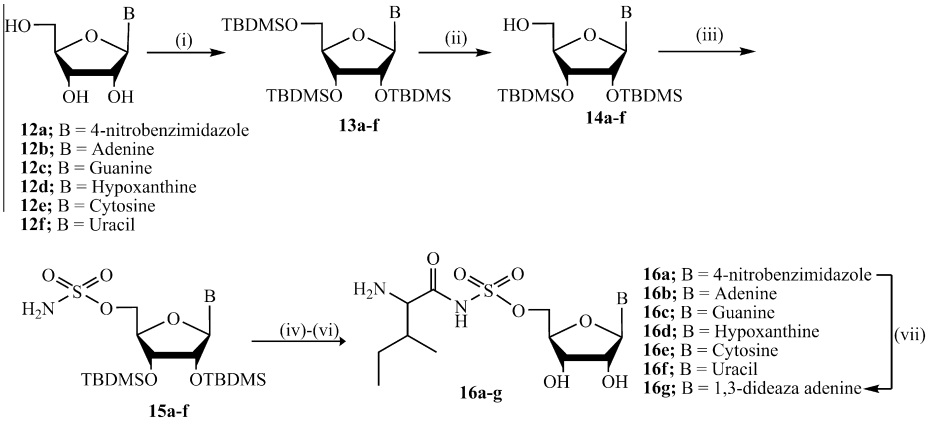
Figure 3. In vitro aminoacylation reaction in presence of the respective aaSA analogues **16a–16g** using S30 cell extract of *E. coli* wt.

3. Biological evaluation

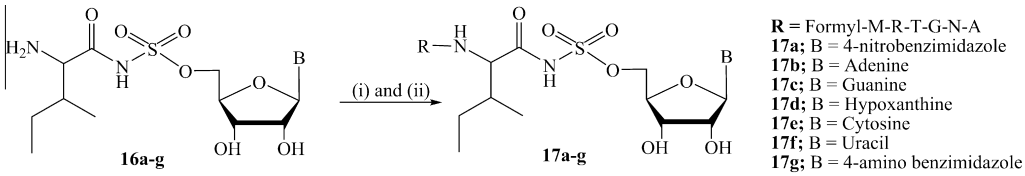
The obtained analogues were evaluated for inhibition of isoleucine incorporation in tRNA under in vitro aminoacylation conditions. Hereto, $tRNA^{Ile}$ aminoacylation reactions were carried out in *E. coli* cell extracts in presence of the respective test compounds, and using radioactively labeled isoleucine as described before (Fig. 3).[27] The remarkable order of inhibitory activity found for the different sulfamates surprisingly showed the strongest inhibition for the U analogue **16f** followed by I > adenine = C > 4-ABI > 4-NBI > G. Excited with the results from our in vitro aminoacylation experiments, we evaluated the analogues **16a–g** for their ability to inhibit the growth of microorganisms by a disc diffusion method (data not shown). In line with previous results[34] none of the analogues showed growth inhibitory activity (maximum concentration tested was 5 mM). Hexapeptidyl (formyl-MRTGNA-OH) conjugates of these analogues likewise were evaluated for their growth inhibitory properties. Unfortunately, none of conjugates **17a–g** displayed any activity against *E. coli* wt (Fig. 5). Upon evaluation with the *E. coli* Ara-Yej inducer strain (BW39758)[34] transient inhibition could be seen after 8 h incubation for the adenosine conjugate **17b** and to a lower extent for the purines **17d** and **17g**. The uracil conjugate **17f** however was devoid of inhibitory activity (Fig. 6).

4. Discussion

Several non-hydrolysable analogues of aa-AMP have been reported in literature as inhibitors of aaRS with the aaSAs displaying nanomolar affinity for the corresponding aaRS in vitro. These inhibitors however lack selectivity for bacterial aaRSs as compared to their human orthologs due to structural similarity with aa-AMP.



Scheme 2. Synthetic scheme for assembly of 5′-*O*-(*N*-isoleucyl)sulfamoyl adenosine and its different base analogues (**16a–g**). Reagents and conditions: (i) TBDMSCl, imidazole, dry DMF, 50 °C, 3 d, 92–98%; (ii) TFA–water, THF, 0 °C, 6 h, 90–99%; (iii) $ClSO_2NCO$, formic acid, DMA, 63–80%; (iv) Boc-Ile-OSu, DBU, DMF, rt, 6 h, (v) TFA:water (5:2), rt, 3 h, (vi) TEA.3HF, THF, rt, overnight 28–65% (over 3 steps); (vii) Pd/C, methanol: water, H_2 atm, rt, 6 h, 40%.



Scheme 3. Strategy for synthesis of the different nucleoside sulfamate-hexapeptidyl conjugates (**17a–g**). Reagents and conditions: (i) DIC, HOBt, DIPEA, DMF, 16 h, (ii) TFA/water/thioanisole (90:7.5:2.5), rt, 2 h, 6–12% (over 2 steps, after HPLC purification).

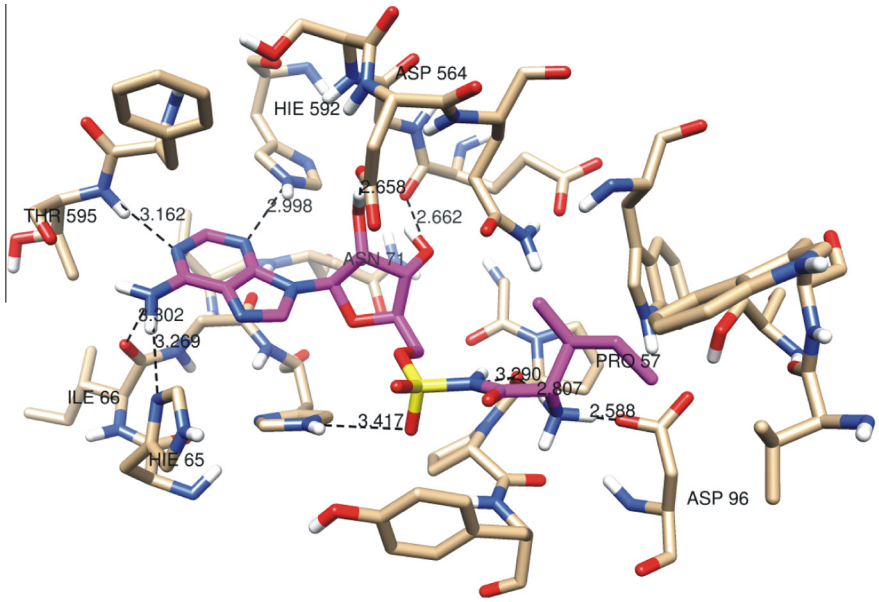


Figure 4. Homology model for *E. coli* IleRS based on the 1PG0 structure (see Section 6.1) including its interactions with the sulfamoylated adenosine analogue **16b**.

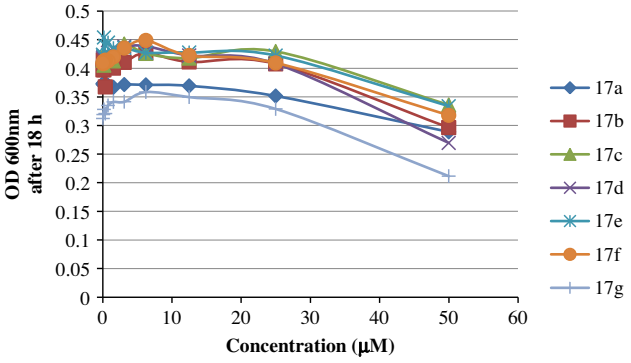


Figure 5. Broth dilution antibacterial activity test against *E. coli* wt in LB medium at different concentrations of the hexapeptidyl conjugates **17a**–**17g**.

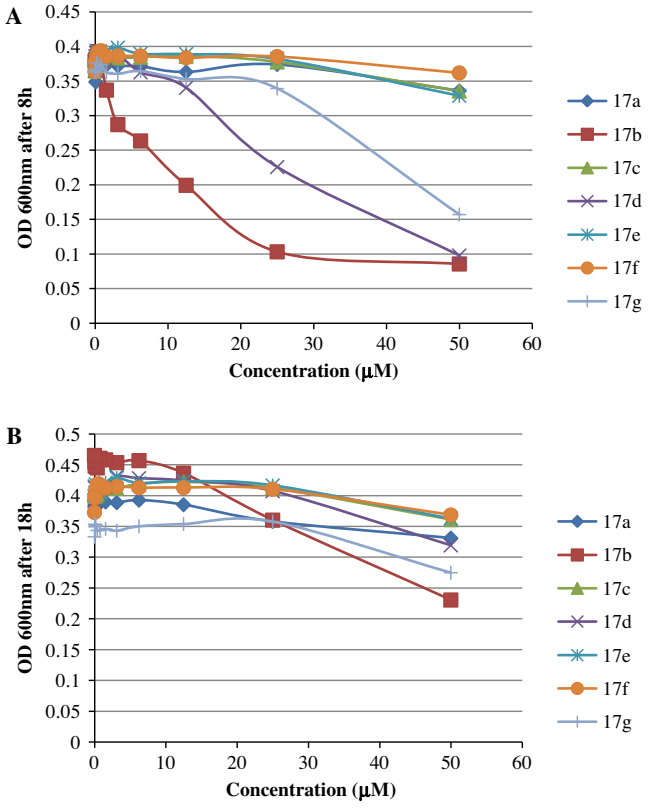


Figure 6. Broth dilution antibacterial test against *E. coli* Ara-Yej (BW39758) strain in LB medium containing 5 mM L-arabinose at different concentrations of the hexapeptidyl conjugates **17a**–**17g** as determined after incubation for 8 h (panel A) and after 18 h (panel B).

Visual inspection of the compounds reported by Cubist Pharmaceuticals, and of albomycin and mupirocin reveals that these structures vary from aaSA analogues in having a heterocyclic base or a modified base or no base moiety at all as in mupirocin, respectively. These observations prompted us to re-investigate the pharmacophoric importance of the adenine base in aaSAs. IleRS, being extensively studied as aaRS target before (e.g. the mupirocin studies and the Cubist Pharmaceuticals inhibitor program), was chosen to study the effect of different bases substituting for adenine. Hereto, isoleucyl-sulfamoyl nucleoside analogues comprising either a natural base like G, I, U or C, or the unnatural base 4-NBI or 4-ABI (the latter corresponding to 1,3-dideaza adenine) were envisaged. The natural nucleobase containing analogues were prepared to investigate the effect of pyrimidine versus purine analogues, whereas the sulfamates containing an unnatural base aimed at evaluating the importance of N^1 and N^3 of the adenine ring, respectively. All analogues were synthesized as outlined in Scheme 2.

From the in vitro aminoacylation experiments it can be concluded that these molecules act as IleRS inhibitors (Fig. 3). The remarkable order of inhibitory activity found for the different sulfamates showed the strongest inhibition for the U analogue (**16f**) followed by I > adenine = C > 4-ABI > 4-NBI > G. This implies that the adenine base or a purine ring per se is not a prerequisite for aaRS inhibition. Furthermore, U and C analogues proved to be either more or equally active respectively, as compared to the adenine base. The latter is rather unexpected in view of their small size occupying only part of the active site. While I is well tolerated, addition of the C-2 amino moiety as in the G analogue resulted in a significant decrease in activity possibly due to steric clashes.

The unnatural 1,3-dideaza-adenine containing analogues displayed lower activity, most probably in view of loss of the reported H-bonding of the adenine N3 position with a His residue of the active site.[35] The 4-NBI analogue (**16a**) proved less active compared to its reduced counterpart, the 4-ABI (1,3-dideaza adenine) analogue suggesting the possible interaction of the amine with the synthetase active site. In contrast, in the reported IleRS·IleAMS of Nakama et al. (with IleAMS being a close analogue to IleSA with nitrogen substituting for the 5′-oxygen), the adenine N6 atom is too far to allow hydrogen bonding.[35]

The strong in vitro inhibition shown by both pyrimidine containing compounds is rather unexpected in view of the omnipresent and more spacious adenosine being used in the majority of biochemical processes. We therefore tried to get some insight into the binding pocket of IleRS, and made an attempt to carry out some molecular simulations with the synthesized inhibitors using a homology modeled IleRS. The *E. coli* IleRS structure being not available in the protein databank, a homology model was created using the I-tasser server[36] starting from the *E. coli* MetRS structure (1PG0); (see Section 6.1). The newly developed inhibitors were then superimposed onto the original inhibitor present in 1PG0 (see Fig. 4).

In using a homology model for our analysis, we cannot use a classical program to dock the inhibitors into the binding site of tRNA synthetase. Numerous amino acid side chains, in addition to the many flexible dihedrals from the inhibitors, would have to be adjusted to accommodate the inhibitors. Unfortunately, the noise on the calculation of the interaction energy values for the different inhibitors proved too large to allow for a clear classification.

Despite the exciting results from the in vitro aminoacylation experiments, no growth inhibitory activity on microorganisms was found for the analogues **16a**–**g** by a disc diffusion method up to maximum concentration tested of 5 mM. This lack of activity corresponds with the general lack of activity in a whole-cell assay as shown before[34] and can be related to limited cell-penetration in accordance with the lack of in vivo efficacy for the IleSA analogue **16b**. Hence with the microcin C strategy in mind, hexapeptidyl conjugates of these analogues were prepared in an effort to improve the uptake of these compounds via a Trojan-horse mechanism. Hence, the conjugates **17a**–**g** were evaluated against *E. coli* wt (Fig. 5) or *E. coli* Ara-Yej inducer (BW39758) strain (Fig. 6) in a broth dilution test. The latter strain upon arabinose induction shows an increased expression of the transporter.[34] Unfortunately, only some transient antibacterial activity was noticed for purine derivatives **17b**, **17d** and **17g** following incubation with the inducer strain, while no antibacterial activity could be recorded for the in vitro strongly active uracil derivative **17f**. As it has been shown previously that the hexapeptide is effectively metabolized by non-specific peptidases[37] failure of uptake is the likely reason for the low activity of these hexapeptidyl conjugates. Hence, being the only different part of these constructs, the heterocyclic base might play an important role for recognition by the transporter as was seen with the tetrazole analogues when coupled to the peptidic carrier.[37]

5. Conclusion

A series of isoleucyl-sulfamoylated nucleoside analogues comprising different base substitutions was synthesized in an effort to evaluate the pharmacophoric importance of the adenine ring for recognition by IleRS. Upon in vitro evaluation in a cellular extract, surprisingly the uracil containing analogue **16f** was shown to be endowed with the highest inhibitory properties. To promote their bacterial uptake, these polar sulfamoylated analogues were converted into their respective hexapeptidyl conjugates in analogy with the natural Trojan-horse antibiotic microcin C. Unfortunately, only weak inhibitory properties could be noticed for the adenosine analogue **17b** and not for the conjugate **17f** comprising the uracil moiety.

6. Experimental section

6.1. Materials and methods

Reagents and solvents were purchased from commercial suppliers (Acros, Sigma–Aldrich, Bachem, Novabiochem) and used as provided, unless indicated otherwise. DMF and THF were of analytical grade and were stored over 4 Å molecular sieves. For reactions involving Fmoc-protected amino acids and peptides, DMF for peptide synthesis (low amine content) was used. 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 with stirring at room temperature, unless indicated otherwise. ^{1}H and ^{13}C NMR spectra of the compounds dissolved in $CDCl_3$, CD_3OD, DMSO-d_6 or D_2O were recorded on a Bruker UltraShield Avance 300 MHz or 500 MHz spectrometer. The chemical shifts are expressed as δ values in parts per million (ppm), using the residual solvent peaks ($CDCl_3$: ^{1}H 7.26 ppm; ^{13}C, 77.16 ppm; DMSO: ^{1}H, 2.50 ppm; ^{13}C, 39.52 ppm; HOD: ^{1}H, 4.79 ppm or 4.975 at 5 °C; CD_3OD: ^{1}H, 3.31 ppm; ^{13}C, 49.00 ppm) as a reference. Coupling constants are given in Hertz (Hz). The peak patterns are indicated by the following abbreviations: bs = broad singlet, d = doublet, m = multiplet, q = quadruplet, s = singlet and t = triplet. Signal assignment for ^{1}H and ^{13}C in the conjugate compounds **17a**–**g** were obtained using [^{1}H,^{1}H]-DQF-COSY,[38] [^{1}H,^{13}C]-HSQC[39] and [^{1}H,^{13}C]-HMBC[40] spectra were recorded on Bruker Avance II 600 with a TCI gradient cryoprobe. 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) at 3 $\mu L \cdot min^{-1}$.

For TLC, precoated aluminium sheets were used (Merck, Silica gel 60 F_{254}). The spots were visualized by UV light at 254 nm. Column chromatography was performed on ICN silica gel 60A 60–200 μm. Final products were purified using a PLRP-S 100 Å column connected to a Merck-Hitachi L6200A Intelligent pump. Eluent compositions are expressed as v/v. Purity was checked by analytical HPLC on a Inertsil ODS-3 (C-18) (4.6 × 100 mm) column, connected to a Shimadzu LC-20AT pump using a Shimadzu SPD-20A UV-detector. Recordings were performed at 254 nm and 214 nm.

6.1.1. 2,1,3-Benzoselenadiazole (7)[29]

An amount of **6** (10.5 g, 97.1 mmol) and selenium dioxide (11.85 g, 106.8 mmol) were refluxed in absolute ethanol (100 mL) for 10 min. The reaction mixture was cooled to rt and ethanol was evaporated in vacuo. The product was precipitated from water to yield 17.6 g (96.1 mmol, 99% yield) of **7** as a faint pink colored solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.46–7.52 (m, 2H, Ar), 7.76–7.83 (m, 2H, Ar). ^{13}C NMR (75 MHz, CD_3OD) δ 124.2, 130.6, 161.6 (C=N). HRMS for $C_6H_5N_2Se$ ([M+H]$^+$) calcd: 184.9612 found 184.9616.

6.1.2. 4-Nitro-2,1,3-benzoselenadiazole (8)[30]

A mixture of 9.1 mL of concd nitric acid and 18.2 mL of concd sulfuric acid was added to a solution of **7** (16.7 g, 91.2 mmol) in concd sulfuric acid (36.5 mL) at 0 °C. The solution was allowed to stir at room temperature for 30 min and then poured into excess ice water. The precipitate was filtered off and washed with distilled water to yield 20.8 g (91.2 mmol, quantitative) of the title compound **8** as yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72–7.78 (dd, 1H, J = 7.5 Hz and 8.7 Hz, H-*meta* to nitro), 8.29

(d, 1H, J = 8.7 Hz, H-*para* to nitro), 8.46 (d, 1H, J = 7.5 Hz, H-*ortho* to nitro). ^{13}C NMR (75 MHz, DMSO-d_6) δ 126.4, 126.8, 129.4, 140.5, 149.9, 159.9. HRMS for $C_6H_4N_3O_2Se$ ([M+H]$^+$) calcd: 229.9463 found 229.9668.

6.1.3. 3-Nitro-1,2-phenylenediamine (9)[32]

To a suspension of **8** (18 g, 79 mmol) in concd hydrochloric acid (225 mL), 70 mL of aqueous hydroiodic acid (57% w/v, 70 mL) was added dropwise at room temperature with vigorous stirring. The reaction mixture was stirred further at room temperature for 3 h. A 5% aqueous sodium hydrogen sulfide solution (400 mL) was added to the dark-red reaction mixture. This was then warmed to 80 °C and filtered hot and afterwards cooled to 4 °C. The needle like salt of the diamine crystallized out. The product was neutralized with 30% NaOH to pH 8 and extracted with ethyl acetate (6 × 100 mL). The ethyl acetate layer was collected, dried over Na_2SO_4, filtered off and evaporated to yield 6.5 g (42.44 mmol, 54%) of the title compound **9** as a red solid: ^{1}H NMR (300 MHz, CD_3OD) δ 6.47–6.56 (dd, 1H, J = 7.5 Hz and 8.7 Hz, Ar), 6.87–6.92 (dd, 1H, J = 1.2 Hz and 7.5 Hz, Ar), 7.48–7.53 (dd, 1H, J = 1.2 Hz and 8.7 Hz, Ar). ^{13}C NMR (75 MHz, CD_3OD) δ 116.4, 117.0, 120.6 (quaternary carbon not detected). HRMS for $C_6H_7N_3O_2Na$ ([M+Na]$^+$) calcd: 176.0431 found 176.1058.

6.1.4. 4(7)-Nitro-benzimidazole (10)[32]

A mixture of **9** (6 g, 39.2 mmol) and triethyl orthoformate (150 mL) was refluxed at 145 °C for 4 h. The solution was evaporated to dryness with a rotary evaporator and the residue obtained was dissolved in formic acid (150 mL) and refluxed for 3 h at 110 °C. When the reaction was completed, the excess formic acid was removed in vacuo and the residue was dissolved in methanol (150 mL) and treated with activated charcoal by overnight stirring at room temperature. The charcoal was removed by filtration through celite and the filtrate was evaporated to dryness. The residue obtained was subjected to silica gel column chromatography to afford 5.54 g (34 mmol, 87%) of **10** as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.43 (t, 1H, J = 8.1 Hz, H-5/6), 8.16 (d, 2H, J = 8.1 Hz, H-4/7 and H-6/5), 8.45 (s, 1H, H-2), 8.47 (br s, 1H, N*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ 118.9 (C-5/6), 121.2 (C-4/7), 126.6 (C-6/5), 145.3 (C-2). HRMS for $C_7H_4N_3O_2$ ([M−H]$^-$) calcd: 162.0309 found 162.0314.

6.1.5. 1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-4-nitrobenzimidazole (11)[32]

The compound **10** (1.2 g, 7.36 mmol) and 1′,2′,3′,5′-tetra-*O*-acetyl-β-D-ribofuranose (2.81 g, 8.83 mmol) were dissolved in 30 mL of dry acetonitrile. A solution of stannic chloride (1 M in DCM, 22.1 mL, 22.1 mmol) was added and the reaction mixture was stirred at 25 °C for 16 h. The reaction mixture was diluted with DCM and then poured under stirring into 30 mL of ice cooled saturated sodium bicarbonate. The resulting suspension was filtered through celite and the layers were separated. The organic layer was further washed with brine and was dried over Na_2SO_4, filtered off and evaporated. The residue was purified by silica gel column chromatography to yield 2.5 g (5.93 mmol, 80%) of the title compound **11** as pale yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.10 (s, 3H, COC*H*$_3$), 2.16 (s, 3H, COC*H*$_3$), 2.18 (s, 3H, COC*H*$_3$), 4.39–4.44 (m, 1H, H-5′a), 4.45–4.50 (m, 1H, H-5′b), 4.51–4.55 (m, 1H, H-4′), 5.51 (t, 1H, J = 5.0 Hz, H-3′), 5.56 (t, 1H, 5.5 Hz, H-2′), 6.15 (d, 1H, J = 5.5 Hz, H-1′), 7.44 (t, 1H, J = 8.0 Hz, H-6), 7.97 (d, 1H, J = 8.0 Hz, H-7), 8.19 (d, 1H, J = 8.0 Hz, H-5), 8.40 (s, 1H, H-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ 19.5 (COC*H*$_3$), 19.6 (COC*H*$_3$), 19.9 (COC*H*$_3$), 61.7 (C-5′), 69.1 (C-3′), 72.5 (C-2′), 79.7 (C-4′), 86.5 (C-1′), 116.3 (C-5), 119.2 (C-7), 122.1 (C-6), 134.2 (C-9), 136.9 (C-4), 138.8 (C-8), 142.8 (C-2), 168.5 (*C*OCH$_3$), 168.7 (*C*OCH$_3$), 169.1 (*C*OCH$_3$). HRMS for $C_{18}H_{19}N_3O_9Na$ ([M+Na]$^+$) calcd: 444.1014 found 444.1012.

6.1.6. 1-β-D-(4-Nitro-benzimidazol-1-yl)-ribofuranoside (12a)

The acylated precursor **11** (2.5 g, 5.93 mmol) was dissolved in methanolic ammonia (7 N, 25 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. Then the mixture was evaporated to dryness and the residue was partitioned between water and ether. The aqueous layer was evaporated to yield 1.4 g (4.74 mmol, 90%) of the title compound as a pale yellow solid which was used further without purification: ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.62–3.66 (m, 1H, H-5′a), 3.67–3.72 (m, 1H, H-5′b), 4.03 (q, 1H, J = 3.0 Hz, H-4′), 4.14 (q, 1H, J = 5.0 Hz, H-3′), 4.38 (q, 1H, J = 6.0 Hz, H-2′), 5.19 (t, 1H, J = 5.5 Hz, 5′-OH), 5.27 (d, 1H, J = 4.5 Hz, 3′-OH), 5.56 (d, 1H, J = 6.0 Hz, H-1′), 7.48 (t, 1H, J = 8.0 Hz, H-6), 8.08 (d, 1H, J = 8.0 Hz, H-7), 8.27 (d, 1H, J = 8.0 Hz, H-5), 8.77 (s, 1H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6) δ 61.1 (C-5′), 70.1 (C-3′), 74.1 (C-2′), 86.0 (C-4′), 89.1 (C-1′), 118.7 (C-7), 118.8 (C-5), 122.3 (C-6), 135.5 (C-9), 136.9 (C-8), 138.8 (C-4), 145.8 (C-2). HRMS for $C_{12}H_{14}N_3O_6$ ([M+H]$^+$) calcd: 296.0877 found 296.0880.

6.1.7. General procedure for synthesis of 2′,3′,5′-tri-*O*-TBDMS nucleosides (13a–f)[41,42]

To a stirred solution of the nucleoside (1 mmol) and imidazole (8 mmol) in DMF (5 mL) was added TBDMSCl (4 mmol). The reaction mixture was stirred at 50 °C for 3 days. After completion of reaction, DMF was evaporated and the residue was partitioned between ethyl acetate (50 mL) and saturated $NaHCO_3$ (50 mL). The layers were separated and the aqueous layer was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with brine, dried over sodium sulfate, filtered and evaporated to yield an oily residue which was purified by silica gel chromatography yield persilyated derivatives in 92–98% yield.

6.1.7.1. 1-β-D-(4-Nitro-benzimidazol-1-yl)-2′,3′,5′-tri-*O*-TBDMS-ribofuranoside (13a). ^{1}H NMR (500 MHz, $CDCl_3$) δ −0.68 (s, 3H, C*H*$_3$-Si), −0.16 (s, 3H, C*H*$_3$-Si), 0.12 (s, 3H, C*H*$_3$-Si), 0.13 (s, 3H, C*H*$_3$-Si), 0.17 (s, 3H, C*H*$_3$-Si), 0.19 (s, 3H, C*H*$_3$-Si), 0.71 (s, 9H, ^{t}Bu C*H*$_3$), 0.96 (s, 9H, ^{t}Bu C*H*$_3$), 0.98 (s, 9H, ^{t}Bu C*H*$_3$), 3.84–3.87 (dd, 1H, J = 2.0 Hz and 11.5 Hz, H-5′a), 3.95–3.99 (dd, 1H, J = 2.0 Hz and 11.5 Hz, H-5′b), 4.16–4.18 (m, 1H, H-4′), 4.23 (d, 1H, J = 4.5 Hz, H-3′), 4.40–4.44 (dd, 1H, J = 4.5 Hz and 7.5 Hz, H-2′), 5.93 (d, 1H, J = 8.0 Hz, H-1′), 7.35 (t, 1H, J = 8.0 Hz, H-6), 8.13 (d, 1H, J = 8.0 Hz, H-7), 8.18 (d, 1H, J = 8.0 Hz, H-5), 8.34 (s, 1H, H-2). ^{13}C NMR (125 MHz, $CDCl_3$) δ −6.7 (*C*H$_3$-Si), −6.3 (*C*H$_3$-Si), −6.2 (*C*H$_3$-Si), −5.5 (*C*H$_3$-Si), −5.4 (*C*H$_3$-Si), −5.3 (*C*H$_3$-Si), 16.9 (^{t}Bu *C*(CH$_3$)$_3$), 17.2 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 24.8 (^{t}Bu, *C*H$_3$), 25.0 (^{t}Bu, *C*H$_3$), 25.21 (^{t}Bu, *C*H$_3$), 62.6 (C-5′), 72.0 (C-3′), 74.9 (C-2′), 86.7 (C-4′), 88.3 (C-1′), 117.8 (C-7), 118.9 (C-5), 121.3 (C-6), 134.7 (C-9), 136.9 (C-8), 138.5 (C-4), 144.2 (C-2). HRMS for $C_{30}H_{56}N_3O_6Si_3$ ([M+H]$^+$) calcd: 638.3471 found 638.3482.

6.1.7.2. 2′,3′,5′-Tri-*O*-TBDMS-guanosine (13c)[43]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.28 (s, 3H, C*H*$_3$-Si), −0.08 (s, 3H, C*H*$_3$-Si), 0.09–0.12 (ms, 12H, 4×C*H*$_3$-Si), 0.73 (s, 9H, ^{t}Bu C*H*$_3$), 0.9 (2s, 18H, ^{t}Bu C*H*$_3$), 3.68–3.73 (dd, 1H, J = 3.9 Hz, and 11.4 Hz, H-5′a), 3.82–3.88 (dd, 1H, J = 5.4 Hz, 11.4 Hz, H-5′b), 3.93–3.95 (m, 1H, H-4′), 4.17 (d, 1H, J = 4.2 Hz, H-3′), 4.56–4.61 (dd, 1H, J = 4.5 Hz and 6.9 Hz, H-2′), 5.74 (d, 1H, J = 7.2 Hz, H-1′), 6.45 (br s, 2H, N*H*$_2$), 7.89 (s, 1H, H-8), 10.61 (br s, N*H*). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.39 (3×CH$_3$-Si), −4.67 (*C*H$_3$-Si), −4.64 (*C*H$_3$-Si), −4.55 (*C*H$_3$-Si), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 17.9 (^{t}Bu *C*(CH$_3$)$_3$), 18.2 (^{t}Bu *C*(CH$_3$)$_3$), 25.6 (^{t}Bu *C*H$_3$), 25.9 (^{t}Bu *C*H$_3$), 26.0 (^{t}Bu *C*H$_3$), 63.1 (C-5′), 72.9 (C-3′), 75.4 (C-2′), 85.4 (C-4′), 85.8 (C-1′), 116.7 (C-5), 135.0 (C-8), 151.8 (C-4),

153.9 (C-2), 156.8 (C-6). HRMS calcd for $C_{28}H_{56}N_5O_5Si_3$ ([M+H]$^+$) calcd: 626.3584 found: 626.3582.

6.1.7.3. 2′,3′,5′-Tri-*O*-TBDMS-inosine (13d)[44]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.53 (s, 3H, C*H*$_3$-Si), −0.13 (s, 3H, C*H*$_3$-Si), −0.02 (s, 3H, C*H*$_3$-Si), 0.01 (2s, 6H, 2×C*H*$_3$-Si), 0.74 (s, 9H, ^{t}Bu, C*H*$_3$), 0.94 (s, 9H, ^{t}Bu, C*H*$_3$), 3.71 (d, 1H, *J* = 12.6 Hz, H-5′), 3.94 (d, 1H, *J* = 12.9 Hz, H-5′), 4.15 (br s, 1H, H-4′), 4.37 (d, 1H, *J* = 4.2 Hz, H-3′), 4.82–4.92 (m, 1H, H-2′), 3.78 (d, 1H, *J* = 7.5 Hz, H-1′), 7.90 (s, 1H, H-8), 8.43 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.7 (*C*H$_3$-Si), −4.52 (*C*H$_3$-Si), −4.47 (*C*H$_3$-Si), −4.4 (*C*H$_3$-Si), 17.9 (^{t}Bu *C*(CH$_3$)$_3$), 18.2 (^{t}Bu *C*(CH$_3$)$_3$), 25.8 (^{t}Bu *C*H$_3$), 25.9 (^{t}Bu *C*H$_3$), 62.9 (C-5′), 73.9 (C-3′), 74.8 (C-2′), 89.2 (C-4′), 91.0 (C-1′), 126.7 (C-5), 140.9 (C-8), 146.3 (C-2), 147.9 (C-4), 159.6 (C-6). HRMS for $C_{28}H_{55}N_4O_5Si_3$ ([M+H]$^+$) calcd: 611.3475 found 611.3472.

6.1.7.4. 2′,3′,5′-Tri-*O*-TBDMS-cytidine (13e)[42]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.01 (s, 6H, 2×C*H*$_3$-Si), 0.06 (s, 3H, C*H*$_3$-Si), 0.07 (s, 3H, C*H*$_3$-Si), 0.09 (s, 3H, C*H*$_3$-Si), 0.10 (s, 3H, C*H*$_3$-Si), 0.83 (s, 9H, ^{t}Bu C*H*$_3$), 0.88 (s, 9H, ^{t}Bu C*H*$_3$), 0.91 (s, 9H, ^{t}Bu C*H*$_3$), 3.67–3.75 (m, 1H, H-5′a), 3.85–3.95 (m, 2H, H-5′b, H-4′), 4.04 (t, 1H, *J* = 4.2 Hz, H-3′), 4.10 (t, 1H, *J* = 4.5 Hz, H-2′), 5.71 (d, 1H, *J* = 7.5 Hz, H-5), 5.82 (d, 1H, *J* = 4.5 Hz, H-1′), 7.17 (br s, 2H, NH$_2$), 7.79 (d, 1H, *J* = 7.5 Hz, H-6). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.7 (*C*H$_3$-Si), −5.6 (*C*H$_3$-Si), −5.0 (2×*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.6 (*C*H$_3$-Si), 17.6 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 18.0 (^{t}Bu *C*(CH$_3$)$_3$), 25.6 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 25.8 (^{t}Bu *C*H$_3$), 61.9 (C-5′), 71.0 (C-3′), 75.2 (C-2′), 83.6 (C-4′), 87.8 (C-5), 93.8 (C-1′), 140.4 (C-6), 155.1 (C-2), 165.4 (C-4). HRMS for $C_{27}H_{56}N_3O_5Si_3$ ([M+H]$^+$) calcd: 586.3522 found 586.3522.

6.1.7.5. 2′,3′,5′-Tri-*O*-TBDMS-uridine (13f). ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.02 (s, 3H, C*H*$_3$-Si), 0.03 (s, 3H, C*H*$_3$-Si), 0.09 (s, 3H, C*H*$_3$-Si), 0.09–0.11 (m, 9H, 3×C*H*$_3$-Si), 0.84 (s, 9H, ^{t}Bu C*H*$_3$), 0.89 (s, 9H, ^{t}Bu C*H*$_3$), 0.91 (s, 9H, ^{t}Bu C*H*$_3$), 3.68–3.75 (dd, 1H, *J* = 2.4 Hz and 11.4 Hz, H-5′a), 3.83–3.90 (dd, 1H, *J* = 3.9 Hz and 11.4 Hz, H-5′b), 3.93–3.97 (m, 1H, H-4′), 4.05–4.09 (m, 1H, H-3′), 4.20–4.25 (m, 1H, H-2′), 5.64 (d, 1H, *J* = 7.8 Hz, H-5), 5.82 (d, 1H, *J* = 5.7 Hz, H-1′), 7.77 (d, 1H, *J* = 8.1 Hz, H-6), 11.40 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.7 (*C*H$_3$-Si), −5.6 (*C*H$_3$-Si), −5.04 (*C*H$_3$-Si), −5.0 (*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.7 (*C*H$_3$-Si), 17.6 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 18.0 (^{t}Bu *C*(CH$_3$)$_3$), 25.5 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 25.8 (^{t}Bu *C*H$_3$), 62.3 (C-5′), 71.7 (C-3′), 74.4 (C-2′), 84.7 (C-4′), 86.9 (C-1′), 101.9 (C-5), 139.9 (C-6), 150.6 (C-2), 162.8 (C-4). HRMS for $C_{27}H_{53}N_2O_6Si_3$ ([M−H]$^-$) calcd: 585.3218 found 585.3218.

6.1.8. General procedure for 5′-desilylation (14a–f)[45]

To a stirred solution of the persilylated nucleoside (200 mg) in THF (4 mL) was added aqueous TFA (2 mL, TFA/water 1:1) at 0 °C. After stirring for 6 h at 0 °C, the reaction mixture was neutralized with saturated aqueous $NaHCO_3$ and diluted with ethyl acetate (80 mL). The layers were separated and the organic phase was washed with water (10 mL) and brine (10 mL), dried over anhydrous Na_2SO_4, filtered and evaporated at reduced pressure. The residue was subjected to silica gel chromatography to provide the 2′,3′-disilylated products as a white solid in 90–99% yield.

6.1.8.1. 1-(β-D-Ribofuranosyl)-4-nitro-2′,3′-di-*O*-TBDMS-benzimidazole (14a). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.54 (s, 3H, C*H*$_3$-Si), −0.09 (s, 3H, C*H*$_3$-Si), 0.15 (s, 6H, 2×C*H*$_3$-Si), 0.72 (s, 9H, ^{t}Bu C*H*$_3$), 1.0 (s, 9H, ^{t}Bu C*H*$_3$), 4.07–4.13 (m, 2H, H-5′a and H-5′b), 4.26 (br s, 1H, H-4′), 4.41 (d, 1H, *J* = 3.3 Hz, H-3′), 4.74–4.80 (dd, 1H, *J* = 4.8 Hz and 7.2 Hz, H-2′), 6.03 (d, 1H, *J* = 6.6 Hz, H-1′), 7.43 (t, 1H, *J* = 8.4 Hz, H-6), 7.92 (d, 1H, *J* = 8.4 Hz, H-7), 8.22 (d, 1H, *J* = 8.1 Hz, H-5), 9.19 (s, 1H, H-2). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.4 (C*H*$_3$-Si), −4.4 (2×C*H*$_3$-Si), −4.3 (C*H*$_3$-Si), 17.9 (^{t}Bu *C*(CH$_3$)$_3$), 18.3 (^{t}Bu *C*(CH$_3$)$_3$), 25.8 (^{t}Bu *C*H$_3$), 26.0 (^{t}Bu *C*H$_3$), 62.0 (C-5′), 74.2 (C-3′), 78.3 (C-2′), 87.9 (C-4′), 89.5 (C-1′), 117.3 (C-7), 120.0 (C-5), 122.6 (C-6), 136.4 (C-9), 136.6 (C-8), 139.1 (C-4), 145.5 (C-2). HRMS for $C_{24}H_{41}N_3O_6Si_2Na$ ([M+Na]$^+$) calcd: 546.2426 found 546.2420.

6.1.8.2. 2′,3′-Di-*O*-TBDMS-adenosine (14b)[46]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.38 (s, 3H, C*H*$_3$-Si), −0.10 (s, 3H, C*H*$_3$-Si), 0.10 (s, 3H, C*H*$_3$-Si), 0.12 (s, 3H, C*H*$_3$-Si), 0.72 (s, 9H, ^{t}Bu C*H*$_3$), 0.92 (s, 9H, ^{t}Bu C*H*$_3$), 3.55–3.63 (m, 1H, H-5′a), 3.70–3.80 (m, 1H, H-5′b), 3.95–4.02 (m, 1H, H-4′), 4.29–4.35 (m, 1H, H-3′), 4.75–4.83 (m, 1H, H-2′), 5.94 (d, 1H, *J* = 6.3 Hz, H-1′), 8.35 (s, 1H, H-2), 8.60 (s, 1H, H-8). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.7 (*C*H$_3$-Si), −4.9 (*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.7 (*C*H$_3$-Si), 17.5 (^{t}Bu *C*(CH$_3$)$_3$), 17.8 (^{t}Bu *C*(CH$_3$)$_3$), 25.5 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 61.0 (C-5′), 72.6 (C-3′), 74.8 (C-2′), 86.9 (C-4′), 87.5 (C-1′), 119.1 (C-5), 141.2 (C-8), 148.6 (C-4), 148.8 (C-2), 153.2 (C-6). HRMS for $C_{22}H_{42}N_5O_4Si_2$ ([M+H]$^+$) calcd: 496.2770 found 496.2771.

6.1.8.3. 2′,3′-Di-*O*-TBDMS-guanosine (14c)[43]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.36 (s, 3H, C*H*$_3$-Si), −0.09 (s, 3H, C*H*$_3$-Si), 0.11 (s, 3H, C*H*$_3$-Si), 0.13 (s, 3H, C*H*$_3$-Si), 0.72 (s, 9H, ^{t}Bu C*H*$_3$), 0.92 (s, 9H, ^{t}Bu C*H*$_3$), 3.53–3.64 (m, 1H, H-5′a), 3.68–3.78 (m, 1H, H-5′b), 3.93–4.0 (m, 1H, H-4′), 4.27–4.32 (m, 1H, H-3′), 4.73 (dd, 1H, *J* = 4.2 Hz and 6.6 Hz, H-2′), 5.24 (t, 1H, *J* = 6.0 Hz, 5′-OH), 5.90 (d, 1H, *J* = 6.9 Hz, H-1′), 8.11 (H-8), 8.39 (NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.7 (*C*H$_3$-Si), −4.84 (*C*H$_3$-Si), −4.76 (*C*H$_3$-Si), −4.7 (*C*H$_3$-Si), 17.5 (^{t}Bu *C*(CH$_3$)$_3$), 17.8 (^{t}Bu *C*(CH$_3$)$_3$), 25.4 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 61.0 (C-5′), 72.7 (C-3′), 75.2 (C-2′), 86.7 (C-4′), 86.8 (C-1′), 124.5 (C-5), 138.9 (C-8), 146.0 (C-4), 148.2 (C-2), 156.5 (C-6). HRMS for $C_{22}H_{42}N_5O_5Si_2$ ([M+H]$^+$) calcd: 512.2718 found 512.2715.

6.1.8.4. 2′,3′-Di-*O*-TBDMS-inosine (14d). ^{1}H NMR (300 MHz, $CDCl_3$) δ −0.53 (s, 3H, C*H*$_3$-Si), −0.13 (s, 3H, C*H*$_3$-Si), −.02 (s, 3H, C*H*$_3$-Si), 0.01 (2s, 6H, 2×C*H*$_3$-Si), 0.74 (s, 9H, ^{t}Bu, C*H*$_3$), 0.94 (s, 9H, ^{t}Bu, C*H*$_3$), 3.71 (d, 1H, *J* = 12.6 Hz, H-5′), 3.94 (d, 1H, *J* = 12.9 Hz, H-5′), 4.15 (br s, 1H, H-4′), 4.37 (d, 1H, *J* = 4.2 Hz, H-3′), 4.82–4.92 (m, 1H, H-2′), 5.78 (d, 1H, *J* = 7.5 Hz, H-1′), 7.90 (s, 1H, H-2), 8.43 (s, 1H, H-8). ^{13}C NMR (75 MHz, $CDCl_3$) δ −5.7 (*C*H$_3$-Si), −4.52 (*C*H$_3$-Si), −4.47 (*C*H$_3$-Si), −4.4 (*C*H$_3$-Si), 17.9 (^{t}Bu *C*(CH$_3$)$_3$), 18.2 (^{t}Bu *C*(CH$_3$)$_3$), 25.8 (^{t}Bu *C*H$_3$), 25.9 (^{t}Bu *C*H$_3$), 62.9 (C-5′), 73.9 (C-3′), 74.8 (C-2′), 89.2 (C-4′), 91.0 (C-1′), 126.7 (C-5), 140.9 (C-8), 146.3 (C-2), 147.9 (C-4), 159.6 (C-6). HRMS for $C_{22}H_{41}N_4O_5Si_2$ ([M+H]$^+$) calcd: 497.2610 found 497.2608.

6.1.8.5. 2′,3′-Di-*O*-TBDMS-cytidine (14e). ^{1}H NMR (600 MHz, DMSO-d_6) δ −0.01 (s, 3H, C*H*$_3$-Si), 0.01 (s, 3H, C*H*$_3$-Si), 0.06 (s, 3H, C*H*$_3$-Si), 0.08 (s, 3H, C*H*$_3$-Si), 0.82 (s, 9H, ^{t}Bu C*H*$_3$), 0.88 (s, 9H, ^{t}Bu C*H*$_3$), 3.51–3.56 (m, 1H, H-5′a), 3.65–3.70 (m, 1H, H-5′b), 3.86 (q, 1H, *J* = 3.6 Hz, H-4′), 4.10 (t, 1H, *J* = 4.2 Hz, H-3′), 4.19 (t, 1H, *J* = 4.8 Hz, H-2′), 5.19–5.21 (m, 1H, 5′-OH) 5.75 (d, 1H, *J* = 7.2 Hz, H-5) 5.77 (d, 1H, *J* = 4.8 Hz, H-1′) 7.20–7.35 (m, 2H, NH$_2$), 7.91 (d, 1H, *J* = 7.2 Hz, H-6). ^{13}C NMR (150 MHz, DMSO-d_6) δ −4.9 (2×C*H*$_3$-Si), −4.8 (C*H*$_3$-Si), −4.6 (C*H*$_3$-Si), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 17.8 (^{t}Bu *C*(CH$_3$)$_3$), 25.7 (^{t}Bu *C*H$_3$), 25.8 (^{t}Bu *C*H$_3$), 60.1 (C-5′), 71.4 (C-3′), 74.9 (C-2′), 84.5 (C-4′), 88.3 (C-5), 94.0 (C-1′), 141.5 (C-6), 155.0 (C-2), 165.2 (C-4). HRMS for $C_{22}H_{42}N_3O_5Si_2$ ([M−H]$^-$) calcd: 472.2657 found 472.2652.

6.1.8.6. 2′,3′-Di-*O*-TBDMS-uridine (14f)[46]. ^{1}H NMR (300 MHz, DMSO-d_6) δ −0.03 (s, 3H, C*H*$_3$-Si), 0.02 (s, 3H, C*H*$_3$-Si), 0.08 (s, 3H, C*H*$_3$-Si), 0.09 (s, 3H, C*H*$_3$-Si), 0.83 (s, 9H, ^{t}Bu C*H*$_3$), 0.88 (s, 9H, ^{t}Bu C*H*$_3$), 3.52–3.70 (m, 2H, H-5′a and H-5′b), 3.85–3.90 (m, 1H, 5′-OH), 4.10–4.17 (m, 1H, H-3′), 4.25 (t, 1H, *J* = 5.7 Hz, H-2′), 5.24 (d, 1H, *J* = 4.8 Hz, H-4′) 5.68 (d, 1H, *J* = 8.4 Hz, H-5) 5.81 (d, 1H,

J = 6.0 Hz, H-1′) 7.93 (d, 1H, J = 8.1 Hz, H-6), 11.34 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.0 (*C*H$_3$-Si), −4.9 (*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.6 (*C*H$_3$-Si), 17.6 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 25.59 (^{t}Bu *C*H$_3$), 25.64 (^{t}Bu *C*H$_3$), 60.4 (C-5′), 71.9 (C-3′), 74.6 (C-2′), 85.5 (C-4′), 86.9 (C-1′), 102.0 (C-5), 140.3 (C-6), 150.8 (C-2), 163.0 (C-4). HRMS for $C_{21}H_{39}N_2O_6Si_2$ ([M−H]$^-$) calcd: 471.2352 found 471.2343.

6.1.9. General procedure for synthesis of the respective 5′-*O*-sulfamoyl nucleosides (15a–f)

Formic acid (2.5 mmol) was added to ice cooled chlorosulfonyl isocyanate (2.5 mmol) and allowed to stir at 0 °C for 5 min. The resulting solid was dissolved in dry acetonitrile (2 mL) and cooled to 0 °C. The obtained sulfamoyl chloride was then added to an ice cooled solution of the respective nucleoside (1 mmol) in dimethyl acetamide (5 mL) and allowed to stir at room temperature for 1 h. After 1 h, TEA (1.5 mL, excess) was added and stirring was continued for an additional 10 min. Subsequently, methanol (2 mL) was added and the mixture was stirred for an additional 15 min. Finally, the solvent was evaporated to dryness and the residue was dissolved in ethyl acetate and washed with saturated $NaHCO_3$. The organic layer was further washed with water and brine. The organic layer was collected, dried over Na_2SO_4, filtered and evaporated. The residue was subjected to silica gel chromatography to afford sulfamoylated nucleoside in 63–80% yield.

6.1.9.1. 1-(2′,3′-Di-*O*-TBDMS)-5′-*O*-sulfamoyl-β-D-ribofuranosyl)-4-nitro-benzimidazole (15a).

^{1}H NMR (500 MHz, CDCl$_3$) δ −0.50 (s, 3H, C*H*$_3$-Si), −0.10 (s, 3H, C*H*$_3$-Si), 0.13 (s, 6H, 2×C*H*$_3$-Si), 0.71 (s, 9H, ^{t}Bu C*H*$_3$), 0.95 (s, 9H, ^{t}Bu C*H*$_3$), 4.31–4.36 (m, 2H, H-5′a and H-5′b), 4.41–4.45 (m, 2H, H-4′, H-3′), 4.55–4.59 (dd, 1H, J = 2.5 Hz, 11.0 Hz, H-2′), 5.97 (d, 1H, J = 6.5 Hz, H-1′), 6.68 (br s, 2H, NH$_2$), 7.40 (t, 1H, J = 8.0 Hz, H-6), 7.85 (d, 1H, J = 8.0 Hz, H-7), 8.15 (d, 1H, J = 8.0 Hz, H-5), 8.59 (s, 1H, H-2). ^{13}C NMR (125 MHz, CDCl$_3$) δ −6.4 (*C*H$_3$-Si), −5.6 (*C*H$_3$-Si), −5.5 (*C*H$_3$-Si), −5.3 (*C*H$_3$-Si), 16.9 (^{t}Bu *C*(CH$_3$)$_3$), 17.1 (^{t}Bu *C*(CH$_3$)$_3$), 24.8 (^{t}Bu *C*H$_3$), 25.0 (^{t}Bu *C*H$_3$), 67.9 (C-5′), 71.9 (C-2′), 76.0 (C-3′), 82.9 (C-4′), 88.0 (C-1′), 116.5 (C-7), 119.1 (C-5), 121.8 (C-6),134.9 (C-9), 135.8 (C-8), 138.1 (C-4), 143.7 (C-2). HRMS for $C_{24}H_{43}N_4O_8SSi_2$ ([M+H]$^+$) calcd: 603.2334 found 603.2325.

6.1.9.2. 2′,3′-Di-*O*-TBDMS-5′-*O*-sulfamoyl-adenosine (15b).

^{1}H NMR (300 MHz, DMSO-d_6) δ −0.37 (s, 3H, C*H*$_3$-Si), −0.08 (s, 3H, C*H*$_3$-Si), 0.13 (s, 3H, C*H*$_3$-Si), 0.15 (s, 3H, C*H*$_3$-Si), 0.71 (s, 9H, ^{t}Bu C*H*$_3$), 0.93 (s, 9H, ^{t}Bu C*H*$_3$), 4.15–4.21 (m, 1H, H-3′), 4.27–4.34 (m, 1H, H-2′), 4.36–4.44 (m, 2H, H-5′a and H-5′b), 4.94–4.94 (dd, 1H, J = 4.5 Hz, 6.6 Hz, H-4′), 5.96 (d, 1H, J = 6.9 Hz, H-1′), 7.31 (s, 2H, NH$_2$), 7.64 (s, 2H, NH$_2$), 8.16 (s, 1H, H-8), 8.36 (s, 1H, H-2). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.6 (*C*H$_3$-Si), −4.9 (*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.7 (*C*H$_3$-Si), 17.5 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 25.4 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 68.0 (C-5′), 72.4 (C-2′), 73.7 (C-3′), 82.8 (C-4′), 87.0 (C-1′), 119.3 (C-5), 139.8 (C-8), 149.4 (C-4), 152.7 (C-2), 156.1 (C-6). HRMS for $C_{22}H_{43}N_6O_6SSi_2$ ([M+H]$^+$) calcd: 575.2498 found 575.2530.

6.1.9.3. 2′,3′-Di-*O*-TBDMS-5′-*O*-sulfamoyl-guanosine (15c).

^{1}H NMR (300 MHz, DMSO-d_6) δ −0.28 (s, 3H, C*H*$_3$-Si), −0.06 (s, 3H, C*H*$_3$-Si), 0.12 (s, 3H, C*H*$_3$-Si), 0.14 (s, 3H, C*H*$_3$-Si), 0.73 (s, 9H, ^{t}Bu C*H*$_3$), 0.92 (s, 9H, ^{t}Bu C*H*$_3$), 4.12–4.17 (m, 1H, H-3′), 4.21–4.35 (m, 3H, H-2′, H-5′a and H-5′b), 4.75–4.81 (dd, 1H, J = 4.5 Hz, 7.2 Hz, H-4′), 5.76 (d, 1H, J = 7.5 Hz, H-1′), 6.47 (br s, 2H, SONH$_2$), 7.66 (s, 2H, 2-NH$_2$), 7.91 (s, 1H, H-8), 10.69 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ −5.6 (*C*H$_3$-Si), −4.9 (*C*H$_3$-Si), −4.7 (2×*C*H$_3$-Si), 17.5 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 25.5 (^{t}Bu *C*H$_3$), 25.7 (^{t}Bu *C*H$_3$), 68.1 (C-5′), 72.7 (C-2′), 74.0 (C-3′), 83.0 (C-4′), 85.8 (C-1′), 116.8 (C-5), 135.6 (C-8), 151.5 (C-4), 153.7 (C-2), 156.7 (C-6). HRMS for $C_{22}H_{43}N_6O_7SSi_2$ ([M+H]$^+$) calcd: 591.2447 found 591.2455.

6.1.9.4. 2′,3′-Di-*O*-TBDMS-5′-*O*-sulfamoyl-inosine (15d).

^{1}H NMR (300 MHz, CDCl$_3$) δ −0.33 (s, 3H, C*H*$_3$-Si), −0.08 (s, 3H, C*H*$_3$-Si), 0.11 (s, 3H, C*H*$_3$-Si), 0.15 (s, 3H, C*H*$_3$-Si),0.76 (s, 9H, ^{t}Bu C*H*$_3$), 0.94 (s, 9H, ^{t}Bu C*H*$_3$), 4.30–4.42 (m, 2H, H-5′a and H-5′b), 4.48–4.52 (m, 1H, H-4′), 4.65–4.75 (m, 1H, H-3′), 4.75–4.80 (m, 1H, H-2′), 5.82 (d, 1H, J = 5.4 Hz, H-1′), 7.01 (br s, 2H, N*H*$_2$), 8.01 (s, 1H, H-8), 8.42 (s, 1H, H-2). ^{13}C NMR (75 MHz, CDCl$_3$) δ −5.1 (*C*H$_3$-Si), −4.7 (*C*H$_3$-Si), −4.6 (*C*H$_3$-Si), −4.3 (*C*H$_3$-Si), 17.9 (^{t}Bu *C*(CH$_3$)$_3$), 18.2 (^{t}Bu *C*(CH$_3$)$_3$), 25.8 (^{t}Bu *C*H$_3$), 26.0 (^{t}Bu *C*H$_3$), 69.0 (C-5′), 72.3 (C-2′), 74.8 (C-3′), 83.3 (C-4′), 90.0 (C-1′), 124.8 (C-5), 140.8 (C-8), 146.1 (C-2), 148.2 (C-4), 158.3 (C-6). HRMS for $C_{22}H_{41}N_5O_7SSi_2$ ([M+H]$^+$) calcd: 576.2338 found 576.2347.

6.1.9.5. 2′,3′-Di-*O*-TBDMS-5′-*O*-sulfamoyl-cytidine (15e).

^{1}H NMR (300 MHz, DMSO-d_6) δ 0.00 (s, 3H, C*H*$_3$-Si), 0.02 (s, 3H, C*H*$_3$-Si), 0.08 (s, 3H, C*H*$_3$-Si), 0.10 (s, 3H, C*H*$_3$-Si),0.83 (s, 9H, ^{t}Bu C*H*$_3$), 0.88 (s, 9H, ^{t}Bu C*H*$_3$), 4.04–4.18 (m, 3H, H-5′a, H-5′b and H-4′), 4.22–4.32 (m, 2H, H-2′ and H-3′), 5.73–5.80 (m, 2H, H-5 and H-1′), 7.21–7.38 (m, 2H, SON*H*$_2$), 7.63 (d, 1H, J = 7.5 Hz, H-6), 7.68 (s, 2H, N*H*$_2$). ^{13}C NMR (75 MHz, , DMSO-d_6) δ −5.0 (*C*H$_3$-Si), −4.9 (*C*H$_3$-Si), −4.8 (*C*H$_3$-Si), −4.6 (*C*H$_3$-Si), 17.6 (^{t}Bu *C*(CH$_3$)$_3$), 17.7 (^{t}Bu *C*(CH$_3$)$_3$), 25.67 (^{t}Bu *C*H$_3$), 25.69 (^{t}Bu *C*H$_3$), 67.5 (C-5′), 71.2 (C-3′), 74.0 (C-2′), 81.0 (C-4′), 88.9 (C-5), 94.3 (C-1′), 141.1 (C-6), 154.8 (C-2), 165.2 (C-4). HRMS for $C_{21}H_{43}N_4O_7SSi_2$ ([M+H]$^+$) calcd: 551.2385 found 551.2380.

6.1.9.6. 2′,3′-Di-*O*-TBDMS-5′-*O*-sulfamoyl-uridine (15f).

^{1}H NMR (300 MHz, CDCl$_3$) δ 0.07 (s, 3H, C*H*$_3$-Si), 0.08 (s, 3H, C*H*$_3$-Si), 0.10 (s, 3H, C*H*$_3$-Si), 0.11 (s, 3H, C*H*$_3$-Si),0.88 (s, 9H, ^{t}Bu C*H*$_3$), 0.91 (s, 9H, ^{t}Bu C*H*$_3$), 4.12 (t, 1H, J = 4.2 Hz, H-5′a) 4.24 (q, 1H, J = 3.9 Hz, H-4′), 4.28 (dd, 1H, J = 3.6 Hz and 11.1 Hz, H-5′b), 4.48–4.54 (m, 2H, H-2′ and H-3′), 5.53 (d, 1H, J = 4.5 Hz, H-1′), 5.62 (br s, 2H, SON*H*$_2$), 5.77 (d, 1H, J = 8.1 Hz, H-5), 7.50 (d, 1H, J = 8.1 Hz, H-6), 9.70 (br s, 1H, N*H*). ^{13}C NMR (75 MHz, CDCl$_3$) δ −5.8 (*C*H$_3$-Si), −5.59 (*C*H$_3$-Si), −5.56 (*C*H$_3$-Si), −5.2 (*C*H$_3$-Si), 17.10 (^{t}Bu *C*(CH$_3$)$_3$), 17.15 (^{t}Bu *C*(CH$_3$)$_3$), 24.90 (^{t}Bu *C*H$_3$), 24.93 (^{t}Bu *C*H$_3$), 67.4 (C-5′), 70.5 (C-3′), 72.6 (C-2′), 81.1 (C-4′), 92.1 (C-1′), 101.6 (C-5), 141.1 (C-6), 149.7 (C-2), 162.7 (C-4). HRMS for $C_{21}H_{41}N_3O_8SSi_2Na$ ([M+Na]$^+$) calcd: 574.2045 found 574.2043.

6.1.10. General procedure for synthesis of 5′-*O*-(*N*-L-isoleucyl)-sulfamoyl nucleosides (16a–f)

To a solution of the respective 2′,3′-di-*O*-(tert-butyldimethylsilyl)-5′-*O*-sulfamoyl nucleoside (1.0 mmol) and N^{α}-Boc-L-isoleucine *N*-hydroxysuccinimide ester (1.1 equiv) in DMF (3 mL) was added DBU (1.1 equiv) and the reaction mixture was stirred at rt for 6–8 h. Next, the volatiles were removed in vacuo and the residue was purified by silica gel chromatography (Et$_3$N 1%, MeOH 2.5–10% in CH_2Cl_2).

The coupled product was next treated with TFA/H_2O (5:2 v/v) for 2 h at rt, after which the volatiles were evaporated and coevaporated twice with EtOH and once with EtOH and Et$_3$N (2 mL), to neutralize any remaining acid. The compound was carefully dried and dissolved in THF (2 mL) and Et$_3$N·3HF (0.5 mL). After 3 h, another 0.4 mL of Et$_3$N·3HF was added and the reaction mixture was stirred further for 22 h. The reaction mixture was evaporated and the residue was purified by flash chromatography (5–50% CH_2Cl_2/MeOH) and finally by HPLC using PLRP-S 100 Å column and acetonitrile/water as mobile phase to yield isoleucyl-sulfamoyl nucleoside as white solid in 28–65% yield.

6.1.10.1. 1-[5′-*O*-(*N*-L-Isoleucyl-sulfamoyl-β-D-ribofuranosyl)]-4-nitro-benzimidazole (16a).

^{1}H NMR (500 MHz, D_2O) δ 0.74 (t, 3H, J = 10.0 Hz, Ile-δ-C*H*$_3$), 0.88 (d, 3H, J = 10.0 Hz, Ile-γ-C*H*$_3$),

1.05–1.15 (m, 1H, Ile-γ-CH_2 Ha), 1.32–1.41 (m, 1H, Ile-γ-CH_2 Hb), 1.79–1.85 (m, 1H, Ile-β-C*H*), 3.46 (d, 1H, *J* = 5.0 Hz, Ile-α-C*H*), 4.38–4.45 (m, 2H, H-5′a and H-5′b), 4.45–4.50 (m, 2H, H-3′ and H-4′), 4.61 (t, 1H, *J* = 5.0 Hz, H-2′), 6.09 (d, 1H, *J* = 5.0 Hz, H-1′), 7.44 (t, 1H, *J* = 10.0 Hz, H-6), 8.03 (d, 1H, *J* = 10.0 Hz, H-7), 8.12 (d, 1H, *J* = 10.0 Hz, H-5) 8.60 (s, 1H, H-2). ^{13}C NMR (75 MHz, D_2O) δ 10.4 (Ile-δ-CH_3), 14.4 (Ile-γ-CH_3), 23.6 (Ile-δ-CH_2), 37.0 (Ile-β-C*H*), 60.3 (Ile-α-C*H*), 67.7 (C-5′), 69.8 (C-3′), 73.5 (C-2′), 82.5 (C-4′), 88.8 (C-1′), 118.7 (C-7), 120.2 (C-5), 123.0 (C-6), 134.5 (C-9), 135.6 (C-8), 137.3 (C-4), 145.0 (C-2), 178.4 (C=O, Ile). HRMS for $C_{18}H_{24}N_5O_9S$ ($[M-H]^-$) calcd: 486.1300 found 486.1301.

6.1.10.2. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-adenosine (16b). ^{1}H NMR (300 MHz, D_2O) δ 0.87 (t, 3H, *J* = 7.5 Hz, Ile-δ-CH_3), 0.98 (d, 3H, *J* = 6.9 Hz, Ile-γ-CH_3), 1.02–1.28 (m, 1H, Ile-γ-CH_2 Ha), 1.40–1.53 (m, 1H, Ile-γ-CH_2 Hb), 1.95–2.07 (m, 1H, Ile-β-C*H*), 3.76 (d, 1H, *J* = 4.2 Hz, Ile-α-C*H*), 4.43–4.51 (m, 3H, H-5′a, H-5′b and H-4′), 4.56 (t, 1H, *J* = 5.1 Hz, H-3′), 4.75–4.78 (m, 1H, merged with D2O peak, H-2′), 6.13 (d, 1H, *J* = 5.1 Hz, H-1′), 8.24 (s, 1H, H-8), 8.42 (s, 1H, H-2). ^{13}C NMR (75 MHz, D_2O) δ 11.5 (Ile-δ-CH_3), 15.1 (Ile-γ-CH_3), 24.7 (Ile-δ-CH_2), 37.0 (Ile-β-C*H*), 60.7 (Ile-α-C*H*), 68.9 (C-5′), 70.8 (C-3′), 74.7 (C-2′), 82.9 (C-4′), 87.9 (C-1′), 119.1 (C-5), 140.4 (C-8), 149.5 (C-4), 153.1 (C-2), 155.8 (C-6), 175.6 (C=O, Ile). HRMS for $C_{16}H_{24}N_7O_7S$ ($[M-H]^-$) calcd: 458.1463 found 458.1461.

6.1.10.3. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-guanosine (16c). ^{1}H NMR (600 MHz, D_2O) δ 0.83 (t, 3H, *J* = 7.2 Hz, Ile-δ-CH_3), 0.94 (d, 3H, *J* = 7.2 Hz, Ile-γ-CH_3), 1.01–1.19 (m, 1H, Ile-γ-CH_2 Ha), 1.34–1.43 (m, 1H, Ile-γ-CH_2 H_b), 1.92–1.99 (m, 1H, Ile-β-C*H*), 3.89 (d, 1H, *J* = 4.2 Hz, Ile-α-C*H*), 4.35–4.40 (m, 3H, H-5′a, H-5′b and H-4′), 4.50 (t, 1H, *J* = 4.2 Hz, H-3′), 4.70–4.75 (m, 1H, H-2′), 5.92 (d, 1H, *J* = 6.0 Hz, H-1′), 8.03 (s, 1H, H-8). ^{13}C NMR (125 MHz, D_2O) δ 10.5 (Ile-δ-CH_3), 14.1 (Ile-γ-CH_3), 23.7 (Ile-δ-CH_2), 36.0 (Ile-β-CH), 59.7 (Ile-α-CH), 68.0 (C-5′), 69.8 (C-3′), 73.2 (C-2′), 81.9 (C-4′), 86.6 (C-1′), 115.9 (C-5), 137.1 (C-8), 151.4 (C-4), 153.6 (C-2), 158.7 (C-6), 174.7 (C=O, Ile). HRMS for $C_{16}H_{24}N_7O_8S$ ($[M-H]^-$) calcd: 474.1412 found 474.1407.

6.1.10.4. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-inosine (16d). ^{1}H NMR (300 MHz, D_2O) δ 0.74 (t, 3H, *J* = 7.5 Hz, Ile-δ-CH_3), 0.86 (d, 3H, *J* = 7.2 Hz, Ile-γ-CH_3), 1.01–1.17 (m, 1H, Ile-γ-CH_2 Ha), 1.25–1.40 (m, 1H, Ile-γ-CH_2 H_b), 1.82–1.95 (m, 1H, Ile-β-C*H*), 3.62 (d, 1H, *J* = 4.2 Hz, Ile-α-C*H*), 4.29–4.38 (m, 3H, H-5′a, H-5′b and H-4′), 4.43 (t, 1H, *J* = 4.2 Hz, H-3′), 4.62–4.70 (m, 1H, merged with solvent peak, H-2′), 6.02 (d, 1H, *J* = 5.1 Hz, H-1′), 8.11 (s, 1H, H-8), 8.27 (s, 1H, H-2). ^{13}C NMR (75 MHz, D_2O) δ 10.6 (Ile-δ-CH_3), 14.1 (Ile-γ-CH_3), 23.7 (Ile-δ-CH_2), 36.1 (Ile-β-C*H*), 59.8 (Ile-α-C*H*), 67.8 (C-5′), 69.9 (C-3′), 73.9 (C-2′), 82.1 (C-4′), 87.4 (C-1′), 123.4 (C-5), 139.2 (C-8), 146.1 (C-2), 148.4 (C-4), 158.5 (C-6), 175.0 (C=O, Ile). HRMS for $C_{16}H_{23}N_6O_8S$ ($[M-H]^-$) calcd: 459.1303 found 459.1304.

6.1.10.5. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-cytidine (16e). ^{1}H NMR (300 MHz, D_2O) δ 0.91 (t, 3H, *J* = 7.5 Hz, Ile-δ-CH_3), 1.02 (d, 3H, *J* = 7.2 Hz, Ile-γ-CH_3), 1.17–1.33 (m, 1H, Ile-γ-CH_2 Ha), 1.41–1.56 (m, 1H, Ile-γ-CH_2 H_b), 1.99–2.10 (m, 1H, Ile-β-C*H*), 3.76 (d, 1H, *J* = 4.2 Hz, Ile-α-C*H*), 4.23–4.37 (m, 4H, H-5′a, H-5′b H-4′and H-3′), 4.44–4.51 (m, 1H, H-2′), 5.95 (d, 1H, *J* = 3.3 Hz, H-1′), 6.10 (d, 1H, *J* = 7.5 Hz, H-5) 7.83 (d, 1H, *J* = 7.8 Hz, H-6). ^{13}C NMR (75 MHz, D_2O) δ 10.6 (Ile-δ-CH_3), 14.2 (Ile-γ-CH_3), 23.8 (Ile-δ-CH_2), 36.1 (Ile-β-CH), 59.8 (Ile-α-CH), 67.6 (C-5′), 68.9 (C-3′), 73.7 (C-2′), 80.9 (C-4′), 89.5 (C-5), 96.1 (C-1′), 141.0 (C-6), 157.3 (C-2), 165.8 (C-4), 174.8 (C=O, Ile). HRMS for $C_{15}H_{26}N_5O_8S$ ($[M+H]^+$) calcd: 436.1496 found 436.1495.

6.1.10.6. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-uridine (16f). ^{1}H NMR (300 MHz, D_2O) δ 0.93 (t, 3H, *J* = 7.5 Hz, Ile-δ-CH_3), 1.02 (d, 3H, *J* = 6.9 Hz, Ile-γ-CH_3), 1.18–1.34 (m, 1H, Ile-γ-CH_2 Ha), 1.42–1.56 (m, 1H, Ile-γ-CH_2 H_b), 1.98–2.11 (m, 1H, Ile-β-C*H*), 3.75 (d, 1H, *J* = 3.9 Hz, Ile-α-C*H*), 4.38–4.48 (m, 5H, H-5′a, H-5′b, H-4′ H-3′, H-2′), 5.92 (m, 2H, H-1′ and H-5), 7.84 (d, 1H, *J* = 8.1 Hz, H-6). ^{13}C NMR (75 MHz, D_2O) δ 10.7 (Ile-δ-CH_3), 14.2 (Ile-γ-CH_3), 23.8 (Ile-δ-CH_2), 36.1 (Ile-β-CH), 59.7 (Ile-α-CH), 67.7 (C-5′), 69.2 (C-3′), 73.3 (C-2′), 81.4 (C-4′), 88.5 (C-1′), 102.2 (C-5), 141.2 (C-6), 151.3 (C-2), 165.8 (C-4), 174.8 (C=O, Ile). HRMS for $C_{15}H_{23}N_4O_9S$ ($[M-H]^-$) calcd: 435.1191 found 435.1192.

6.1.11. 5′-*O*-(*N*-L-Isoleucyl)-sulfamoyl-1,3-dideaza-adenosine (16g)

To a solution of **16a** (50 mg, 0.1 mmol) in dry methanol (5 mL) was added Pd/C (10% w/w, 20 mg) and the mixture was stirred under H_2 atmosphere at room temperature for 7 h. Next, the catalyst was filtered off and the solvent was evaporated to dryness. The product was purified by silica gel column chromatography and finally by HPLC to yield 19 mg (0.04 mmol, 40%) of title compound as faint brown solid.

^{1}H NMR (500 MHz, D_2O) δ 0.80 (t, 3H, *J* = 7.5 Hz, Ile-δ-CH_3), 0.95 (d, 3H, *J* = 7.0 Hz, Ile-γ-CH_3), 1.12–1.24 (m, 1H, Ile-γ-CH_2 Ha), 1.37–1.46 (m, 1H, Ile-γ-CH_2 Hb), 1.95–2.02 (m, 1H, Ile-β-C*H*), 3.71 (d, 1H, *J* = 4.0 Hz, Ile-α-C*H*), 4.41–4.46 (m, 3H, H-5′a, H-5′b, H-4′), 4.48–4.51 (m,1H, H-3′), 4.67 (t, 1H, *J* = 6.0 Hz, H-2′), 6.04 (d, 1H, *J* = 6.5 Hz, H-1′), 6.78 (d, 1H, *J* = 7.5 Hz, H-1), 7.17 (d, 1H, *J* = 7.5 Hz, H-3), 7.23 (t, 1H, *J* = 8.0 Hz, H-2), 8.34 (s, 1H, H-8). ^{13}C NMR (125 MHz, D_2O) δ 10.5 (Ile-δ-CH_3), 14.1 (Ile-γ-CH_3), 23.7 (Ile-δ-CH_2), 36.0 (Ile-β-CH), 59.8 (Ile-α-CH), 68.1 (C-5′), 69.7 (C-3′), 72.8 (C-2′), 82.0 (C-4′), 88.2 (C-1′), 101.7 (C-3), 108.3 (C-1), 124.7 (C-2), 132.1 (C-5), 133.0 (C-6), 137.5 (C-4), 140.5 (C-8), 174.8 (C=O Ile). HRMS for $C_{18}H_{26}N_5O_7S$ ($[M-H]^-$) calcd: 456.1558 found 456.1560.

6.1.12. General procedure for synthesis of nucleoside sulfamate–hexapeptidyl conjugates (17a–g)

The peptide formyl-methionyl-arginyl(2,2,4,6,7-pentamethyl dihydrobenzofuran-5-sulfonyl)-threonyl(tBu)-glycyl-asparaginyl-(trityl)-alanyl-OH was synthesized on a 2-chlorotrityl chloride resin using standard Fmoc-based solid phase peptide chemistry. The protected hexapeptide was cleaved from the resin using a mixture of HOAc/trifluoroethanol/DCM (1:1:8, v/v) in 30 min. Following RP-HPLC purification, the peptide (20 mg, 16.13 μmol, 1.0 equiv) and HOBt (9 mg, 64.52 μmol, 4.0 equiv) were dissolved in DMF (500 μL) and DIC (10 μL, 64.52 μmol, 4.0 equiv) was added. This mixture was stirred for 1 h at rt under argon atmosphere. Following addition of DIPEA (7.5 μL, 40.33 μmol, 2.5 equiv), the mixture was added to the nucleoside sulfamate analogue **16a–g** (32.26 μmol, 2.0 equiv) and stirred for 16 h at rt under argon. Next, the volatiles were evaporated and the residue was taken up in a mixture of CH_3CN/water. This was purified on a PoraPak Rxn RP 6cc Vac Cartridge 80 μm particle (Waters®) column with a CH_3CN gradient of 25–100% in water). The fractions containing the product were evaporated and the protected conjugate was subsequently deprotected using a mixture of 90% TFA, 7.5% H_2O and 2.5% thioanisole. The reaction mixture was poured in chilled diethyl ether and centrifuged. Supernatant was decanted and the residue was re-suspended in chilled diethyl ether and centrifuged. A small amount of TEA (500 μL) was added for final washing. The product was dissolved in CH_3CN/water and purified by RP-HPLC (solvent A: 25 mM TEAB in H_2O; solvent B: 25 mM TEAB in CH_3CN; see supporting file for HPLC analysis of all final compounds). Overall yield for coupling, deprotection and tedious HPLC purification is generally low and affords about 6–12% yield.

6.1.12.1. fMRTGNAI-S(4-nitrobenzimidazole) (17a). ^{1}H NMR (600 MHz, D_2O) δ 0.73 (t, 3H, J = 7.2 Hz, Ile-δ-CH_3), 0.81 (d, 3H, J = 6.6 Hz, Ile-γ-CH_3), 1.00–1.10 (m, 1H, Ile-γ-CH_2, Ha), 1.14–1.21 (m, Thr-γ-CH_3, TEA-CH_3), 1.27–1.39 (d+m, 4H, J = 7.2 Hz, Ala-β-CH_3 and Ile-γ-CH_2, Hb), 1.55 (m, 2H, Arg-γ-CH_2), 1.67–1.88 (m, 3H, Ile β-CH and 2H, Arg-β-CH_2), 1.95–2.07 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.53 (m, 2H, Met-γ-CH_2), 2.37 (dd, 1H, J = 8.4 Hz and 15.6 Hz, Asn-β-CH_2, Ha), 2.78 (dd, 1H, J = 4.8 Hz, and 16.2 Hz, Asn-β-CH_2, Hb), 3.00 (m, TEA-CH_2), 3.10 (m, 2H, Arg-δ-CH_2), 3.87 (d, 1H, J = 16.9 Hz, Gly-α-CH_2, Ha), 3.94–4.00 (m, 2H, Ile-α-CH and Gly-α-CH_2, Hb), 4.19–4.28 (m, 2H, Thr-β-CH, Ala-α-CH), 4.31–4.41 (m, 4H, Thr-α-CH, Arg-α-CH and H-5′a and H-5′b), 4.42–4.46 (m, 2H, H-3′and H-4′), 4.49 (dd, 1H, J = 5.9 Hz and 8.2 Hz, Met-α-CH), 4.64–4.68 (m, 2H, H-2′ and Asn-α-CH), 6.13 (d, 1H, J = 6.7 Hz, H-1′), 7.54 (t, 1H, J = 8.3 Hz, H-6), 8.08 (s, 1H, CHO), 8.16 (d, 1H, J = 8.3 Hz, H-7), 8.26 (d, 1H, J = 8.3 Hz, H-5), 8.68 (s, 1H, H-2). ^{13}C NMR (150 MHz, D_2O) δ 8.9 (TEA-CH_3), 10.1 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.7 (Ile-γ-CH_3), 16.1 (Ala-β-CH_3), 18.3 (Thr-γ-CH_3), 24.0 (Ile-γ-CH_2), 24.1 (Arg-γ-CH_2), 27.7 (Arg-β-CH_2), 28.7 (Met-γ-CH_2), 30.1 (Met-β-CH_2), 35.9 (Asn-β-CH_2), 36.6 (Ile-β-CH), 40.1 (Arg-δ-CH_2), 42.2 (Gly-α-CH_2), 45.6 (TEA-CH_2), 49.5 (Ala-α-CH), 49.9 (Asn-α-CH), 51.1 (Met-α-CH), 53.0 (Arg-α-CH), 58.6 (Thr-α-CH), 60.2 (Ile-α-CH), 66.9 (Thr-β-CH), 68.0 (C-5′), 70.0 (C-3′), 73.4 (C-2′), 82.8 (C-4′), 88.7 (C-1′), 119.0 (C-7), 120.5 (C-5), 123.2 (C-6), 134.8 (C-8), 135.9 (C-9), 137.7 (C-4), 145.4 (C-2), 156.2 (Arg-Cζ), 163.9 (CHO), 170.9 (Gly-CO), 171.8 (Asn-CO), 172.0 (Thr-CO), 173.0 (Met-CO), 173.3 (Arg-CO), 174.0 (Ala-CO and Asn-Cγ), 179.1 (Ile-CO). HRMS for $C_{43}H_{66}N_{15}O_{18}S_2$ ([M−H]$^-$) calcd: 1144.4158 found 1144.4150.

6.1.12.2. *f*MRTGNAI-SA (17b). ^{1}H NMR (600 MHz, D_2O) δ 0.85 (t, 3H, J = 7.2 Hz, Ile-δ-CH_3), 0.90 (d, 3H, J = 6.6 Hz, Ile-γ-CH_3), 1.13–1.24 (m, Thr-γ-CH_3, TEA-CH_3 and Ile-γ-CH_2, Ha), 1.36 (d, 3H, J = 7.2 Hz, Ala-β-CH_3), 1.43–1.52 (m, 1H, Ile-γ-CH_2, Hb), 1.61 (m, 2H, Arg-γ-CH_2), 1.71–1.92 (m, 3H, Ile β-CH and 2H, Arg-β-CH_2), 1.96–2.08 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.55 (m, 2H, Met-γ-CH_2), 2.70 (dd, 1H, J = 8.4 Hz and 15.6 Hz, Asn-β-CH_2, Ha), 2.79 (dd, 1H, J = 4.8 Hz, and 16.2 Hz, Asn-β-CH_2, Hb), 3.08–3.21 (m, TEA-CH_2 and Arg-δ-CH_2), 3.97 (d, 1H, J = 16.9 Hz, Gly-α-CH_2, Ha), 3.98 (d, 1H, J = 16.9 Hz, Gly-α-CH_2, Hb), 4.06 (d, 1H, J = 6.9 Hz, Ile-α-CH), 4.21 (m, 1H, Thr-β-CH), 4.26–4.46 (m, 7H, Ala-α-CH, Thr-α-CH, Arg-α-CH, H-3′, H-4′ and H-5′a and H-5′b), 4.50 (dd, 1H, J = 5.9 Hz, and 8.2 Hz, Met-α-CH), 4.67 (dd, 1H, J = 5.2 Hz and 8.9 Hz, Asn-α-CH), 4.72 (m, 1H, H-2′), 6.09 (d, 1H, J = 5.9 Hz, H-1′), 8.08 (s, 1H, CHO), 8.22 (s, 1H, H-2), 8.42 (s, 1H, H-8). ^{13}C NMR (150 MHz, D_2O) δ 7.9 (TEA-CH_3), 9.8 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.4 (Ile-γ-CH_3), 16.0 (Ala-β-CH_3), 18.4 (Thr-γ-CH_3), 24.1 (Ile-γ-CH_2), 24.3 (Arg-γ-CH_2), 27.6 (Arg-β-CH_2), 28.7 (Met-γ-CH_2), 30.1 (Met-β-CH_2), 35.4 (Asn-β-CH_2), 35.9 (Ile-β-CH), 40.1 (Arg-δ-CH_2), 42.1 (Gly-α-CH_2), 46.3 (TEA-CH_2), 49.5 (Ala-α-CH), 50.0 (Asn-α-CH), 51.1 (Met-α-CH), 53.2 (Arg-α-CH), 58.8 (Thr-α-CH), 60.4 (Ile-α-CH), 66.9 (Thr-β-CH), 68.0 (C-5′), 70.2 (C-3′), 73.7 (C-2′), 82.8 (C-4′), 86.5 (C-1′), 139.4 (C-8), 148.8 (C-4), 152.7 (C-2), 155.2 (C-6), 156.4 (Arg-Cζ), 163.9 (CHO), 170.9 (Gly-CO), 172.0 (Asn-CO and Thr-CO), 173.1 (Met-CO), 173.4 (Arg-CO), 174.0 (Ala-CO and Asn-Cγ), 179.6 (Ile-CO). HRMS for $C_{41}H_{68}N_{17}O_{16}S_2$ ([M−H]$^-$) calcd: 1116.4320 found 1116.4305.

6.1.12.3. *f*MRTGNAI-SG (17c). ^{1}H NMR (600 MHz, D_2O) δ 0.77 (t, 3H, J = 7.2 Hz, Ile-δ-CH_3), 0.82 (d, 3H, J = 6.6 Hz, Ile-γ-CH_3), 1.05 (m, 1H, Ile-γ-CH_2, Ha), 1.16 (d, J = 6.3 Hz, 3H, Thr-γ-CH_3), 1.24 (t, J = 3.6 Hz, TEA-CH_3), 1.32 (d, 3H, J = 7.2 Hz, Ala-β-CH_3), 1.36 (m, 1H, Ile-γ-CH_2, Hb), 1.58 (m, 2H, Arg-γ-CH_2), 1.69–1.89 (m, 3H, Ile β-CH and Arg-β-CH_2), 1.94–2.08 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.45–2.57 (m, 2H, Met-γ-CH_2), 2.70 (dd, 1H, J = 8.4 Hz and 15.6 Hz, Asn-β-CH_2, Ha), 2.80 (dd, 1H, J = 4.8 Hz and 16.2 Hz, Asn-β-CH_2, Hb), 3.11 (t, 2H, J = 6.6 Hz, Arg-δ-CH_2), 3.16 (q, J = 7.8 Hz, TEA-CH_2), 3.89 (d, 1H, J = 6.6 Hz and 16.6 Hz, Gly-α-CH_2, Ha), 3.95–4.02 (m, 2H, Ile-α-CH and Gly-α-CH_2, Hb), 4.18–4.24 (m, 1H, Thr-β-CH), 4.25–4.31 (m, 3H, H-5′a and H-5′b, Ala-α-CH), 4.33–4.37 (m, 2H, J = 4.4 Hz, Thr-α-CH, H-4′), 4.37–4.43 (m, 2H, Arg-α-CH, H-3′), 4.49 (dd, 1H, J = 5.9 Hz and 8.2 Hz, Met-α-CH), 4.66–4.72 (m, 2H, Asn-α-CH and H-2′), 5.89 (d, 1H, J = 6.6 Hz, H-1′), 8.00 (s, 1H, H-8), 8.08 (s, 1H, CHO). ^{13}C NMR (150 MHz, D_2O) δ 7.3 (TEA-CH_3), 10.0 (Ile-δ-CH_3), 13.6 (Met-SCH_3), 14.5 (Ile-γ-CH_3), 15.9 (Ala-β-CH_3), 18.2 (Thr-γ-CH_3), 23.8 (Ile-γ-CH_2), 23.9 (Arg-γ-CH_2), 27.7 (Arg-β-CH_2), 28.7 (Met-γ-CH_2), 29.8 (Met-β-CH_2), 35.8 (Asn-β-CH_2), 36.5 (Ile-β-CH), 40.1 (Arg-δ-CH_2), 42.1 (Gly-α-CH_2), 46.0 (TEA-CH_2), 49.5 (Ala-α-CH), 49.9 (Asn-α-CH), 51.1 (Met-α-CH), 53.0 (Arg-α-CH), 58.6 (Thr-α-CH), 60.2 (Ile-α-CH), 66.9 (Thr-β-CH), 68.1 (C-5′), 70.0 (C-3′), 73.1 (C-2′), 82.0 (C-4′), 85.9 (C-1′), 116.2 (C-5), 136.3 (C-8), 151.4 (C-4), 156.0 (Arg-Cζ), 163.7 (CHO), 170.6 (Gly-CO), 171.5 (Asn-CO), 171.7 (Thr-CO), 173.1 (Met-CO), 173.2 (Arg-CO), 173.7 (Ala-CO), 173.8 (Asn-Cγ), 179.1 (Ile-CO). HRMS for $C_{41}H_{66}N_{17}O_{17}S_2$ ([M-H]$^-$) calcd: 1132.4269 found 1132.4274.

6.1.12.4. fMRTGNAI-SI (17d). ^{1}H NMR (600 MHz, D_2O) δ 0.75 (t, 3H, J = 7.2 Hz, Ile-δ-CH_3), 0.81 (d, 3H, J = 6.6 Hz, Ile-γ-CH_3), 1.04–1.10 (m, Ile-γ-CH_2, Ha, TEA-CH_3), 1.16 (d, 3H, J = 6.3 Hz, Thr-γ-CH_3),1.29–1.37 (d+m, 4H, J = 7.2 Hz, Ala-β-CH_3 and Ile-γ-CH_2, Ha), 1.55 (m, 2H, Arg-γ-CH_2), 1.69–1.77 (m, 2H, Ile-β-CH and Arg-β-CH_2, Ha), 1.79–1.87 (m, 1H, Arg-β-CH_2, Hb), 1.94–2.08 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.45–2.57 (m, 2H, Met-γ-CH_2), 2.67–2.76 (m, TEA-CH_2 and Asn-β-CH_2, Ha), 2.80 (dd, 1H, J = 4.8 Hz and 16.2 Hz, Asn-β-CH_2, Hb), 3.10 (t, 2H, J = 6.6 Hz, Arg-δ-CH_2), 3.87 (d, 1H, J = 16.8 Hz, Gly-α-CH_2, Ha), 3.95–4.01 (m, 2H, Ile-α-CH and Gly-α-CH_2, Hb), 4.18–4.24 (m, 1H, Thr-β-CH), 4.25–4.31 (m, 3H, H-5′a and H-5′b, Ala-α-CH), 4.33 (d, 1H, J = 4.4 Hz, Thr-α-CH), 4.36–4.43 (m, 3H, Arg-α-CH, H-3′, H-4′), 4.48 (dd, 1H, J = 5.9 Hz and 8.2 Hz, Met-α-CH), 4.67–4.73 (m, 2H, Asn-α-CH and H-2′), 6.03 (d, 1H, J = 6.6 Hz, H-1′), 8.08 (s, 1H, CHO), 8.11 (s, 1H, H-2), 8.25 (s, 1H, H-8). ^{13}C NMR (150 MHz, D_2O) δ 9.1 (TEA-CH_3), 10.1 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.7 (Ile-γ-CH_3), 16.1 (Ala-β-CH_3), 18.4 (Thr-γ-CH_3), 24.2 (Ile-γ-CH_2 and Arg-γ-CH_2), 27.5 (Arg-β-CH_2), 28.8 (Met-γ-CH_2), 30.0 (Met-β-CH_2), 35.9 (Asn-β-CH_2), 36.6 (Ile-β-CH), 40.0 (Arg-δ-CH_2), 42.2 (Gly-α-CH_2), 45.5 (TEA-CH_2), 49.6 (Ala-α-CH), 50.0 (Asn-α-CH), 51.3 (Met-α-CH), 53.2 (Arg-α-CH), 58.8 (Thr-α-CH), 60.4 (Ile-α-CH), 66.9 (Thr-β-CH), 68.2 (C-5′), 70.3 (C-3′), 73.5 (C-2′), 82.2 (C-4′), 86.1 (C-1′), 123.0 (C-5), 137.3 (C-8), 149.8 (C-4), 153.6 (C-2), 156.2 (Arg-Cζ), 164.0 (CHO), 167.3 (C-6), 170.9 (Gly-CO), 171.8 (Asn-CO), 172.1 (Thr-CO), 173.3 (Met-CO), 173.6 (Arg-CO), 174.0 (Ala-CO), 174.1 (Asn-Cγ), 179.4 (Ile-CO). HRMS for $C_{41}H_{65}N_{16}O_{17}S_2$ ([M−H]$^-$) calcd: 1117.4160 found 1117.4186.

6.1.12.5. *f*MRTGNAI-SC (17e). ^{1}H NMR (600 MHz, D_2O) δ 0.82 (t, 3H, J = 7.2 Hz, Ile-δ-CH_3), 0.88 (d, 3H, J = 6.6 Hz, Ile-γ-CH_3), 1.07–1.15 (m, 1H, Ile-γ-CH_2, Ha), 1.15–1.24 (m, Thr-γ-CH_3 and TEA-CH_3), 1.35 (d, 3H, J = 7.2 Hz, Ala-β-CH_3), 1.37–1.44 (m, 1H, Ile-γ-CH_2, Hb), 1.61 (m, 2H, Arg-γ-CH_2), 1.75 (m, 1H, Ile-β-CH), 1.84 (m, 2H, Arg-β-CH_2), 1.96–2.09 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.55 (m, 2H, Met-γ-CH_2), 2.70 (m, 1H, Asn-β-CH_2, Ha), 2.80 (dd, 1H, J = 4.8 Hz and 16.2 Hz, Asn-β-CH_2, Hb), 3.07 (m, TEA-CH_2), 3.16 (t, 2H, J = 6.6 Hz, Arg-δ-CH_2), 3.89 (d, 1H, J = 5.4 Hz, Gly-α-CH_2, Ha), 3.95–4.04 (m, 2H, Ile-α-CH and Gly-α-CH_2, Hb), 4.18–4.25 (m, 2H, H-2′ and Thr-β-CH), 4.27–4.33 (m, 4H, H-5′a, H-5′b, H-3′ and Ala-α-CH), 4.34–4.45 (m, 3H, Thr-α-CH, Arg-α-CH and H-4′), 4.50 (dd, 1H, J = 5.9 Hz and 8.2 Hz, Met-α-CH), 4.68 (dd, 1H, J = 5.2 Hz and 8.9 Hz, Asn-α-CH), 5.92 (d, 1H, J = 3.2 Hz, H-1′), 6.03 (d, 1H, J = 7.5 Hz, H-5), 7.82 (d, 1H, J = 7.5 Hz, H-6), 8.09 (s, 1H, CHO). ^{13}C NMR (150 MHz, D_2O) δ 8.3

(TEA-CH_3), 10.3 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.8 (Ile-γ-CH_3), 16.2 (Ala-β-CH_3), 18.4 (Thr-γ-CH_3), 24.2 (Ile-γ-CH_2 and Arg-γ-CH_2), 27.5 (Arg-β-CH_2), 28.8 (Met-γ-CH_2), 30.0 (Met-β-CH_2), 35.9 (Asn-β-CH_2), 36.7 (Ile-β-CH), 40.1 (Arg-δ-CH_2), 42.2 (Gly-α-CH_2), 46.0 (TEA-CH_2), 49.6 (Ala-α-CH), 50.0 (Asn-α-CH), 51.3 (Met-α-CH), 53.2 (Arg-α-CH), 58.8 (Thr-α-CH), 60.4 (Ile-α-CH), 66.9 (Thr-β-CH), 67.2 (C-5′), 69.0 (C-3′), 73.8 (C-2′), 81.1 (C-4′), 89.1 (C-1′), 96.2 (C-5), 140.9 (C-6), 156.3 (Arg-Cζ), 157.4 (C-4), 164.0 (CHO), 165.8 (C-2), 170.9 (Gly-CO), 171.9 (Asn-CO), 172.1 (Thr-CO), 173.4 (Met-CO), 173.6 (Arg-CO), 174.1 (Ala-CO and Asn-Cγ), 179.4 (Ile-CO). HRMS for $C_{40}H_{66}N_{15}O_{17}S_2$ ([M−H]$^-$) calcd: 1092.4208 found 1092.4196.

6.1.12.6. *f*MRTGNAI-SU (17f). ^{1}H NMR (600 MHz, D_2O) δ 0.83 (t, 3H, *J* = 7.2 Hz, Ile-δ-CH_3), 0.89 (d, 3H, *J* = 6.6 Hz, Ile-γ-CH_3), 1.07–1.16 (m, 1H, Ile-γ-CH_2, Ha), 1.18 (d, 3H, *J* = 8.4 Hz, Thr-γ-CH_3), 1.19–1.27 (t, *J* = 3.6 Hz, TEA-CH_3), 1.36 (d, 3H, *J* = 7.2 Hz, Ala-β-CH_3), 1.37–1.44 (m, 1H, Ile-γ-CH_2, Hb), 1.61 (m, 2H, Arg-γ-CH_2), 1.7–1.92 (m, 3H, Ile-β-CH and 2H, Arg-β-CH_2), 1.98–2.09 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.55 (m, 2H, Met-γ-CH_2), 2.70 (dd, 1H, *J* = 8.4 Hz and 15.6 Hz, Asn-β-CH_2, Ha), 2.80 (dd, 1H, *J* = 4.8 Hz, and 16.2 Hz, Asn-β-CH_2, Hb), 3.17 (m, TEA-CH_2 and Arg-δ-CH_2), 3.89 (d, 1H, *J* = 16.9 Hz, Gly-α-CH_2, Ha), 3.98 (d, 1H, *J* = 16.9 Hz, Gly-α-CH_2, Hb), 4.01 (d, 1H, *J* = 6.9 Hz, Ile-α-CH), 4.19–4.37 (m, 8H, Thr-β-CH, Ala-α-CH, Thr-α-CH, Arg-α-CH, H-3′, H-4′, H-5′a-and H-5′b), 4.41 (dd, 1H, *J* = 5.9 Hz and 8.2 Hz, Met-α-CH), 4.51 (dd, 1H, *J* = 6.0 Hz, and 8.3 Hz, H-2′), 4.68 (dd, 1H, *J* = 5.2 Hz and 8.9 Hz, Asn-α-CH), 5.88 (d, 1H, *J* = 7.6 Hz, H-5), 5.94 (d, 1H, *J* = 5.3 Hz, H-1′), 7.79 (d, 1H, *J* = 7.6 Hz, H-6), 8.09 (s, 1H, CHO). ^{13}C NMR (150 MHz, D_2O) δ 7.9 (TEA-CH_3), 10.2 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.8 (Ile-γ-CH_3), 16.2 (Ala-β-CH_3), 18.4 (Thr-γ-CH_3), 24.2 (Ile-γ-CH_2), 24.3 (Arg-γ-CH_2), 27.6 (Arg-β-CH_2), 28.8 (Met-γ-CH_2), 30.0 (Met-β-CH_2), 35.9 (Asn-β-CH_2), 36.6 (Ile-β-CH), 40.1 (Arg-δ-CH_2), 42.2 (Gly-α-CH_2), 46.3 (TEA-CH_2), 49.5 (Ala-α-CH), 50.0 (Asn-α-CH), 51.3 (Met-α-CH), 53.2 (Arg-α-CH), 58.8 (Thr-α-CH), 60.4 (Ile-α-CH), 66.8 (Thr-β-CH), 67.6 (C-5′), 69.4 (C-3′), 73.4 (C-2′), 81.5 (C-4′), 88.2 (C-1′), 102.4 (C-5), 140.8 (C-6), 156.3 (Arg-Cζ), 152.8 (C-2), 164.0 (CHO), 167.4 (C-4), 170.9 (Gly-CO), 171.9 (Asn-CO), 172.1 (Thr-CO), 173.3 (Met-CO), 173.6 (Arg-CO), 174.0 (Ala-CO and Asn-Cγ), 179.5 (Ile-CO). HRMS for $C_{40}H_{65}N_{14}O_{18}S_2$ ([M−H]$^-$) calcd: 1093.4048 found 1093.4047.

6.1.12.7. *f*MRTGNAI-S(4-ABI) (17g). ^{1}H NMR (600 MHz, D_2O) δ 0.76 (t, 3H, *J* = 7.2 Hz, Ile-δ-CH_3), 0.84 (d, 3H, *J* = 6.6 Hz, Ile-γ-CH_3), 1.05–1.11 (m, 1H, Ile-γ-CH_2, Ha), 1.16 (d, 4H, *J* = 6.3 Hz, 3H, Thr-γ-CH_3), 1.22 (t, *J* = 3.6 Hz, TEA-CH_3), 1.30 (d, 3H, *J* = 7.2 Hz, Ala-β-CH_3), 1.36–1.40 (m, 1H, Ile-γ-CH_2, Hb), 1.51–1.60 (m, 2H, Arg-γ-CH_2), 1.60–1.72 (m, 1H, Ile-β-CH), 1.77–1.85 (m, 2H, Arg-β-CH_2), 1.94–2.08 (m+s, 5H, Met-β-CH_2 and Met-SCH_3), 2.47–2.58 (m, 2H, Met-γ-CH_2), 2.97 (dd, 1H, *J* = 8.4 Hz and 15.6 Hz, Asn-β-CH_2, Ha), 2.78 (dd, 1H, *J* = 4.8 Hz, and 16.2 Hz, Asn-β-CH_2, Hb), 3.05–3.15 (m, Arg-δ-CH_2 and TEA-CH_2), 3.87 (dd, 1H, *J* = 6.6 Hz and 16.6 Hz, Gly-α-CH_2, Ha), 3.94–4.03 (m, 2H, Ile-α-CH and Gly-α-CH_2, Hb), 4.17–4.23 (m, 1H, Thr-β-CH), 4.23–4.29 (m, 1H, Ala-α-CH), 4.30–4.43 (m, 6H, Thr-α-CH, Arg-α-CH, H-3′, H-4′, H-5′a and H-5′b), 4.48 (t, 1H, *J* = 7.2 Hz, Met-α-CH), 4.64–4.68 (m, 2H, Asn-α-CH and H-2′), 5.99 (d, 1H, *J* = 6.0 Hz, H-1′), 6.73 (d, 1H, *J* = 7.2 Hz, H-5), 7.15–7.21 (d+tr, 2H, H-6, and H-7), 8.01 (s, 1H, CHO), 8.31 (s, 1H, H-2). ^{13}C NMR (150 MHz, D_2O) δ 8.0 (TEA-CH_3), 10.1 (Ile-δ-CH_3), 13.8 (Met-SCH_3), 14.8 (Ile-γ-CH_3), 16.1 (Ala-β-CH_3), 18.4 (Thr-γ-CH_3), 24.2 (Ile-γ-CH_2 and Arg-γ-CH_2), 27.5 (Arg-β-CH_2), 28.8 (Met-γ-CH_2), 30.0 (Met-β-CH_2), 35.9 (Asn-β-CH_2), 36.6 (Ile-β-CH), 40.0 (Arg-δ-CH_2), 42.2 (Gly-α-CH_2), 46.2 (TEA-CH_2), 49.6 (Ala-α-CH), 49.9 (Asn-α-CH), 51.3 (Met-α-CH), 53.2 (Arg-α-CH), 58.7 (Thr-α-CH), 60.4 (Ile-α-CH), 66.9 (Thr-β-CH), 68.1 (C-5′), 70.0 (C-3′), 72.6 (C-2′), 82.3 (C-4′), 88.2 (C-1′), 102.1 (C-7), 108.5 (C-5), 124.8 (C-6), 132.2 (C-9), 133.0 (C-8) 137.4 (C-4), 140.7 (C-2), 156.2 (Arg-Cζ), 164.0 (CHO), 170.9 (Gly-CO), 171.8 (Asn-CO), 172.1 (Thr-CO), 173.3 (Met-CO), 173.6 (Arg-CO), 174.0 (Ala-CO), 174.1 (Asn-Cγ), 179.4 (Ile-CO). HRMS for $C_{43}H_{68}N_{15}O_{16}S_2$ ([M−H]$^-$) calcd: 1114.4415 found 1114.4412.

6.2. Model building and analysis

With the *E. coli* Ile-tRNA synthetase structure being not available in the protein databank, a homology model was created using the I-tasser server.[36] The sequence of the enzyme was taken from the uniprot database (enzyme code EC = 6.1.1.5 and uniprot id P00956). As single 3D template structure, we used the *E. coli* methionyl-tRNA synthetase structure (1PG0) belonging to the same class I of tRNA synthetases. This structure contains a methionyl adenylate analogue inhibitor similar to the seven inhibitors used in this study, and therefore was used as template for our adenylate inhibitor. Additionally, the threonyl sulfamoyl adenosine inhibitor found in structure 3UH0[47] was used to complete part of the sulfamoyl tail in our inhibitor structures. The six inhibitors having different bases were built by superimposing the respective base onto the adenosine using quatfit. The homology model of *E. coli* Ile-tRNA synthetase was then superimposed onto structure 1PG0 using dali.[48]

All seven inhibitors were then superimposed onto the original inhibitor present in 1PG0 resulting in seven complexes of *E. coli* Ile-tRNA synthetase with inhibitor. As no interactions were seen between the base atoms of the pyridine inhibitors and the enzyme pocket, the uridine and cytosine inhibitors were repositioned with the base coinciding with the six-membered ring of the adenine base. The complexes were prepared for use in the AMBER software.[49] The parameters for enzyme structure were taken from the ff99bsc0 force field.[50] The seven ligand molecules were parameterized with Antechamber using the gaff force field.[51] Some patches were introduced to the parameters to get the right conformation for the base atoms and the sulfamoyl group after energy minimization. The molecular mechanics energy of the complexes was minimized using 500 steps. Then the mm/pbsa method[52] was applied on the minimized complexes to obtain the binding energies for the different inhibitors. Visual inspection[53] together with a ligplot analysis[54] was used for interpretation.

6.3. Biological activity experiments

6.3.1. Whole cell activity determinations

The respective bacteria were grown overnight in Luria Broth (LB) medium and cultured again the following day in fresh LB medium or LB-medium containing 5 mM L-arabinose. Compounds were titrated in a 96-well plate using either LB-medium ±5 mM L-arabinose to dilute the compounds. To each well, 85 μL LB-medium ±5 mM L-arabinose was added to a total volume of 90 μL. Next, 10 μL of bacterial cell culture grown to a OD_{600} of 0.1 was added. The cultures were next placed into a Tecan Infinite M200® incubator and shaken at 37 °C. The OD_{600} was determined after 8 and 18 h, respectively. The broth dilution tests were performed in duplicate.

Bacterial strains used for the evaluations: *E. coli* Ara-Yej (BW39758), expressing the YejABEF transporter upon L-arabinose induction; *E. coli* wt used as wild type control. The antibacterial activities of all compounds were determined by monitoring the optical density of suspensions of cell-cultures.

6.3.2. Aminoacylation experiments

To assess the degree of inhibition of the aminoacylation reaction, in vitro tests were performed using the relevant S30 cell extracts.

Preparation of S30 cell extracts. Cells were grown in 50 mL LB-medium. After centrifugation at 3000*g* for 10 min the supernatant was discarded and the pellet was resuspended in 40 mL buffer containing: Tris·HCl or HEPES·KOH (pH = 8.0; 20 mM), $MgCl_2$ (10 mM), KCl (100 mM). The cell suspension was centrifuged again at 3000*g*. This procedure was repeated twice. The pellet was resuspended in 1 mL of the following buffer Tris·HCl or HEPES·KOH (pH = 8.0; 20 mM), $MgCl_2$ (10 mM), KCl (100 mM), DTT (1 mM) and kept at 0 °C. Subsequently, the cells were sonicated for 10 s and left at 0 °C for 10 min. This procedure was repeated 5–8 times. The lysate was centrifuged at 15,000*g* for 30 min at +4 °C.

tRNA aminoacylation reaction: To 1 μL of solution containing inhibitor, 3 μL of *E. coli* S30 extracts was added. Next, 16 μL of the following aminoacylation mixture was added: Tris·HCl (30 mM, pH 8.0), DTT (1 mM), bulk of *E. coli* tRNA (5 g/L), ATP (3 mM), KCl (30 mM), $MgCl_2$ (8 mM), and the specified, ^{14}C-radio-labeled amino acid (40 μM, 200 μCi/mmol). The reaction products were precipitated in cold 10% TCA on Whatman 3 mm papers, 5 min. after the aminoacylation mixture was added. The aminoacylation reaction was carried out at room temperature. Depending on whether or not processing was needed, variable time intervals were included between the addition of the cell extract and the addition of the aminoacylation mixture. After thorough washing with cold 10% TCA, the papers were washed twice with acetone and dried on a heating plate. Following the addition of scintillation liquid (12 mL), the amount of radioactivity was determined in a Tri-card 2300 TR liquid scintillation counter. ^{14}C-Radiolabeled amino acids and scintillation liquid were purchased from Perkin Elmer.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.03.040.

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