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Synthesis of 8-aminoadenosine 5′-(aminoalkyl phosphates), analogues of aminoacyl adenylates

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Abstract—A short and efficient route for the synthesis of aminoalkyl 8-aminoadenylates, potential aminoacyl-tRNA synthetase inhibitors, is presented. Aminoalkyl 8-aminoadenylates were synthesized using a 5′-*H*-phosphonate strategy involving minimal protecting group manipulations and a single final deprotection step.

1. Introduction

Translation of the genetic code and protein synthesis from amino acids takes place in the ribosomes where aminoacyl-tRNAs act as key intermediates. The tRNAs are loaded with the specific amino acids by the action of aminoacyl-tRNA synthetases. There are specific aminoacyl-tRNA synthetases for each amino acid, which means 20 different enzymes for the common 20 amino acids.[1] The aminoacylation reaction, that is, 'charging' of tRNA is carried out in two steps. The first step is the formation of aminoacyl adenylates (I, Fig. 1) by a specific reaction involving amino acids, adenosine triphosphate, and an aminoacyl-tRNA synthetase. The amino acids are thus activated and subsequently transferred to their corresponding tRNA through a highly specific reaction catalyzed by the same enzymes.

Aminoalkyl adenylates (**II**) are inhibitors of these enzymes and are thought to occupy the binding site for the aminoacyl adenylate.[2] This class of compounds is valuable as inhibitors of amino acid activation and as stable substrates for studies of enzyme–substrate interaction. For example, the L-methioninyl adenylate inhibits methionyl-tRNA synthetase in *Escherichia coli* which results in the blocking of acylation of $tRNA^{Met}$, whereas acylation of other tRNAs remains unaffected.[3]

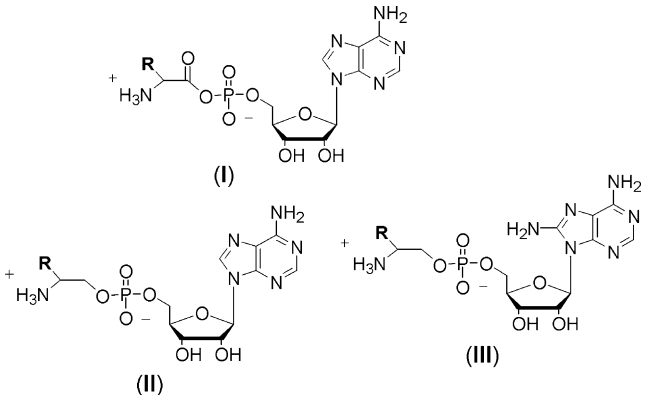

Figure 1. Aminoacyl adenylate **I** and analogues **II** and **III**.

It has also been reported that adenosine has an inhibitory effect on ATP-PP_i exchange and the aminoacylation of tRNA catalyzed by methionyl-tRNA synthetase from *E. coli*.[4] This inhibition is greatly influenced by substitution in the 8-position, as shown by studies with modified adenosines. 8-Aminoadenosine displays a K_i 300 times lower than that of adenosine.[4] It has been suggested that with the 8-aminoadenosine, the 8-position of the imidazole ring comes in close proximity to the functional group

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of an amino acid residue in the enzyme, which can form a strong hydrogen bond with the 8-amino function.[4]

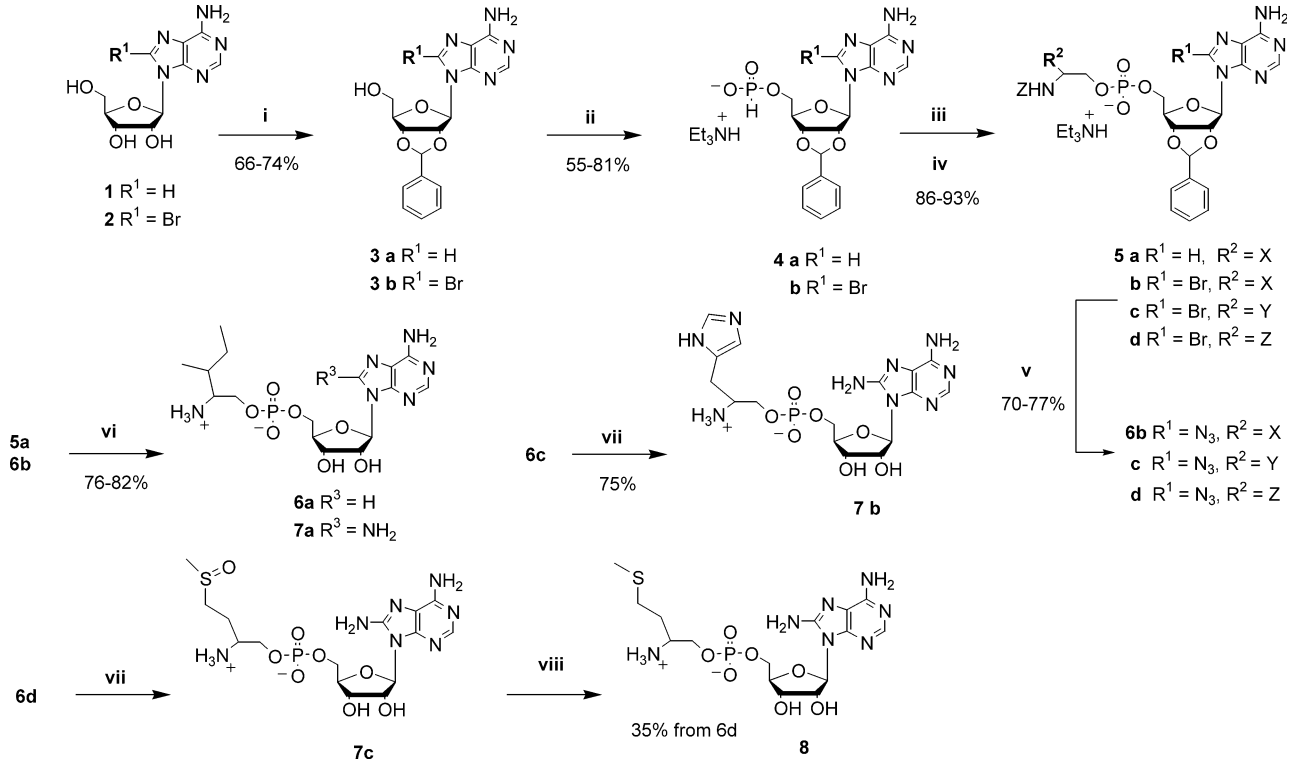
The inhibitory effects found with 8-aminoadenosine and aminoalkyl adenylates suggest that it may be fruitful to combine these modifications in order to obtain more efficient inhibitors of tRNA acylation. Thus, we decided to develop a route for synthesis of the nucleopeptide analogues, aminoalkyl 8-aminoadenylates (**III**).

The synthesis of nucleopeptide analogues is still a challenge due to the variety of functional groups that can be involved. A chemical synthesis of some aminoalkyl adenylates containing unmodified adenosine has been published[2] as well as a method that involves enzyme catalyzed condensation of adenosine-5′-pyrophosphate with the amino alcohol.[5] However, there are no reports on the preparation of aminoalkyl 8-aminoadenylates (**III**). In the present study, we report an efficient and short route for synthesis of aminoalkyl 8-aminoadenylates involving minimal protecting group manipulations and a single final deprotection step.

2. Results and discussion

Aminoalkyl 8-aminoadenylates (**III**) unlike aminoalkyl adenylates (**II**) contain an additional amino function on the nucleobase. We chose to start from adenosine (**1**) and to introduce the amino group at the 8-position via the 8-bromo derivatives **2** (Scheme 1). The latter can be converted to the azido intermediates **6b–d**, which upon reduction should afford the desired 8-amino adducts. For the introduction of the phosphodiester bond between the 5′-hydroxy of the nucleoside and the amino alcohol we selected the *H*-phosphonate approach because of its experimental simplicity, efficiency of generation of *H*-phosphonate mono- and diesters and stability of the monoester intermediate during prolonged storage. Moreover, the reagents used in this approach do not affect the nucleobase and hence, masking of the exocyclic amino group on the purine is not mandatory. Apart from this, a key element in the route towards the preparation of compounds (**III**) is the single step removal of the protecting groups, which will be accompanied by simultaneous reduction of the 8-azido to the 8-amino function. We selected the benzyloxycarbonyl (Z) group for the amino function of the amino alcohol component (and the imidazole moiety of histidinol) and the benzylidene group for the *cis*-diol function of the adenosine. Both groups can be removed by hydrogenolysis, which is also a suitable method for conversion of the azido function into an amine.

As a reference compound, an aminoalkyl adenylate containing unmodified adenosine (**II**) was also synthesized following the same general route, but without the manipulations involving the 8-position. The synthesis of this compound will be described first. In the first step, masking of 2′- and 3′-OH of adenosine was done in order to selectively phosphonylate the 5′-OH function. A benzylidene acetal is a commonly used protecting



Scheme 1. Reagents: (i) benzaldehyde, $ZnCl_2$; (ii) 1. phosphonic acid, Piv-Cl, pyridine; 2. Et_3N–H_2O; (iii) protected amino alcohol, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (OXP); (iv) I_2/pyridine/H_2O; (v) NaN_3, DMSO; (vi) Pd–C, 80% acetic acid; (vii) Pd-black, 80% acetic acid, (viii) $Na_2S_2O_4$. **X** = $CH(CH_3)CH_2CH_3$, **Y** = CH_2(imidazole), and **Z** = $CH_2CH_2SCH_3$.

group for 1,2- and 1,3-diols in carbohydrate chemistry and there are different routes towards the benzylidene protection but α,α-dimethoxytoluene or benzaldehyde are the most used reagents for this purpose.[6] In the first attempt to protect the *cis*-diol system, α,α-dimethoxytoluene was used. An isomeric mixture of products, for example, 2′,3′- and 3′,5′-protected adenosine, was obtained, and due to the low yield of the desired 2′–3′ isomer, alternative conditions, that is, benzaldehyde in the presence of $ZnCl_2$[7,8] were implemented. After stirring compound **1** for three days, in a mixture of benzaldehyde and $ZnCl_2$ at ambient temperature and purification by silica gel chromatography, the protected compound **3a** was obtained in good yield.

The next step was to establish the proper phosphonylation conditions. The free amino group of adenosine can react with a variety of electrophiles and therefore it is often necessary to mask it during synthesis, for example, during phosphoryl-, phosphityl- and phosphonylation processes.[9] However, a few examples of the *O*-selective phosphonylation of *N*-unprotected nucleosides have been reported. Phosphonylation reagents containing the P–H function such as pyrophosphonate[10] and diphenyl *H*-phosphonate[11–13] were completely inert towards the exocyclic amino groups of the naturally occurring nucleosides.[9] The reaction of **3a** with diphenyl *H*-phosphonate (7 equiv) in pyridine, proceeded fast and efficiently (complete in less than 20 min), to give after addition of triethylamine in water compound **4a**. However, when following this procedure, difficulties during work-up and purification were encountered, resulting in a low yield of purified **4a**. These purification problems were probably caused by the similar solubilities and chromatographic mobility of **4a** and the side product from the phosphonylation reagent (i.e., phenyl *H*-phosphonate monoester). This experience led us to adopt an alternative phosphonylation reagent, that is, pyrophosphonate. Pyrophosphonate can be generated practically as a single species from phosphonic acid in pyridine in the presence of 0.5 equiv of pivaloyl chloride.[10] The reaction of equimolar amounts of the pyrophosphonate and the nucleoside proved to be slow, but using a higher concentration of the reagent gave faster and more efficient conversion. Thus, when **3a** was phosphonylated using 0.4 M (2.5 equiv) of pyrophosphonate reagent the reaction was complete within 4 h giving compound **4a** in high yield after purification by silica gel chromatography. No reaction on the unprotected amino group was detected.

Introduction of the Z group for protection of the amino moiety of the amino alcohol was achieved by following a standard peptide chemistry protocol for building block synthesis[14] giving (N^{α}-benzyloxycarbonyl-L-methioninol (**10**) and N^{α}-benzyloxycarbonyl-L-isoleucinol (**9**)) in nearly quantitative yields (Fig. 2). For L-histidinol both the α-amino and the τ-amino functions were protected in one step to give the N^{α},N^{τ}-dibenzyloxycarbonyl-L-histidinol (**11**). With the properly protected amino alcohols (**9**–**11**) and phosphonylated adenosine (**4a**) in hand, we then focused our attention on the condensation step. It has been established that condensing reagents such as pivaloyl chloride[15] and 1-adamantanecarbonyl chloride,[16] which are commonly used in the *H*-phosphonate approach[15,17–21] can react with unprotected amino groups of the nucleoside.[22] Although no reaction was found with the adenine base, we thought that for generality of the method, another condensing agent would be a better alternative. It has been reported that bis (2-oxo-oxazolin-3-yl) phosphonic chloride (OXP), first used in peptide chemistry for peptide bond formation[23] and later in the *H*-phosphonate method for introduction of a phosphodiester linkage,[24,25] does not react readily with the exocyclic amino groups of all common nucleosides.[9] Condensation of amino alcohol **9** with compound **4a** under the agency of OXP in pyridine proceeded smoothly giving, after oxidation with iodine in aqueous pyridine, compound **5a**. After purification, deprotection of the Z and the benzylidene groups was affected by catalytic hydrogenolysis. Thus, treatment of the isoleucinol containing **5a** with palladium–carbon in 80% acetic acid solution for 24 h afforded completely deprotected adenosine 5′-(L-isoleucinyl phosphate) (**6a**) in 82% yield after purification.

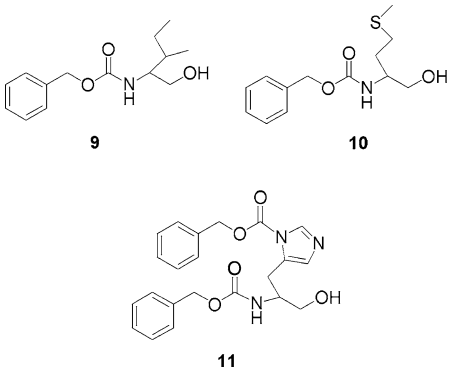


Figure 2. Protected amino alcohols.

Synthesis of L-isoleucinol, L-histidinol and L-methioninol containing **III** also started from adenosine. In the first step, adenosine was converted into 8-bromoadenosine (**2**) with aqueous bromine in acetate buffer (pH 4).[26] Introduction of the benzylidene group for protection of the *cis*-diol function was performed as described above for adenosine (**1**). Phosphonylation of protected 8-bromoadenosine was affected using phosphorous acid/pivaloyl chloride in pyridine under conditions identical to those for the preparation of **4a**. The Z-protected amino alcohols were used together with OXP in the condensation step affording after oxidation and purification the diesters **5b**–**d** in high yields.

Conversion of 8-bromopurines directly to the 8-amino counterparts has been reported[27] but is usually unsuccessful with adenosine derivatives. Reaction with ammonia was unsuccessful below 125 °C and considerable decomposition was observed at higher temperatures. Reaction with hydrazine, which with 8-bromoguanosine gives good yield of 8-aminoguanosine, was unsuccessful with 8-bromoadenosine and produced mainly 8-hydrazinoadenosine. Instead, the most efficient method to the 8-aminoadenosine was via reduction of 8-azidoadenosine.[27] In our synthesis strategy towards aminoalkyl adenylates this

reduction can be accomplished simultaneously to removal of the Z and benzylidene protection, thus obviating the need for an additional step.

Compounds **5b–d** were treated with sodium azide in dimethylsulfoxide at 65 °C during 16 h to give, after work-up and purification, homogeneous compounds **6b–d**. The final stage of the preparation of aminoalkyl-8-aminoadenylates was catalytic hydrogenolysis on palladium. Thus, treatment of the L-isoleucinol containing **6b** using palladium–carbon in 80% acetic acid solution for 24 h afforded completely deprotected L-isoleucinyl 8-aminoadenylate (**7a**) in a yield of 76% after purification. Using palladium–carbon for deprotection/reduction of compounds containing methionine or histidine is often troublesome and the procedure applied for **6b** was unsuccessful when adopted to **6c** and **d**. Prolonged exposure (three days) to the conditions described above for **5a** and **6b** did not result in the conversion of significant amounts of **6c** and **d** into the corresponding **7b** and **8**. To circumvent this problem, the more reactive catalyst palladium black[28] was selected, which has been used successfully for the hydrogenolysis of the Z group from the amino acids L-methionine and L-histidine.[29] Consequently, L-histidinol and L-methioninol derivatives (**6c** and **d**) were dissolved in 80% acetic acid and subjected to treatment with palladium black in a H_2-saturated atmosphere. As a result of this, compound **7b** was isolated after work-up and purification in a yield of 75%. Although palladium black catalyzed hydrogenation has been reported to be successful with methionine derivatives we faced problems with the L-methioninol derivative. It is not unlikely that this is due to poisoning of the catalyst and difficulties with washing out of the product from the catalyst due to adsorbance of the thioether to the palladium. However, we noticed that addition of thiophenol helped in washing out of the product and additional treatment with 80% acetic acid ensured more complete removal of the benzylidine group. In addition, we always observed some accompanying oxidation of the thioether, although thorough flushing of all solutions with nitrogen was performed, and we could also isolate the corresponding sulfoxide (**7c**) in minor quantities. To simplify the isolation of the pure non-oxidized methioninol derivative **8**, we also introduced a sodium hydrosulfite (dithionite) treatment in the work-up scheme. This additional reduction step can conveniently be executed without intermediate purification of **7c**. By taking the above findings into account we could modify the procedure so that hydrogenolysis of **6d** and subsequent treatment with dithionite gave a reasonably acceptable isolated yield (35%) of L-methioninyl-8-aminoadenylate (**8**).

In conclusion, aminoalkyl (L-isoleucinyl and L-histidinyl) 8-aminoadenylates and aminoalkyl (L-isoleucinyl) adenylate were synthesized in high yields. L-Methioninyl-8-aminoadenylate could also be produced although the yield is here limited by problems in final deprotection. The general procedure involves few steps without protection of the nucleobase and with a single deprotection step which simultaneously produces the 8-amino function. The above method should be applicable for the preparation of other aminoalkyl 8-aminoadenylates, since most side chains can be protected with Z (Lys and Trp), as benzyl esters (Asp and Glu) or benzyl ethers (Ser, Thr and Tyr), all of which can be readily removed by hydrogenolysis. At the moment, evaluation of the biological activity of L-isoleucinyl, L-methioninyl and L-histidinyl 8-aminoadenylates is in progress.

3. Experimental

3.1. Materials and methods

All commercial reagents were of synthesis grade and used without further purification. 8-Bromoadenosine[26] (**2**) and 2′,3′-*O*,*O*-benzylidene-adenosine[7,8] (**3a**) were synthesized using published procedures. Pyridine (Labscan) and DMSO were dried and stored over 4 Å molecular sieves. THF was distilled at atmospheric pressure from CaH_2. Dichloromethane, chloroform and methanol were of pro analysis commercial quality. The reactions were monitored by thin-layer chromatography (TLC) analysis using silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV-light. Silica gel chromatography was performed on Matrix silica (60 Å, 35–70 μm, Amicron). General mass spectrometry analysis was performed using electrospray quadropole time-of-flight mass spectrometry (Q-TOF) and accurate mass was determined using electrospray time-of-flight mass spectrometry (Micromass LCT, ESI-TOF). NMR-spectra were obtained on a Bruker AVANCE DRX-400 instrument (400 MHz in ^1H, 100 MHz in ^{13}C and 162 MHz in ^{31}P). Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane, with the residual nondeuterated solvent signal as an internal standard. ^{31}P chemical shifts are calibrated with respect to H_3PO_4 (1% v/v) in D_2O.

3.2. 2′,3′-*O*,*O*-Benzylidene-8-bromoadenosine (3b)

2′, 3′-*O*,*O*-Benzylidene-8-bromoadenosine (**3b**) was synthesized using a similar procedure as for compound **3a**.[7,8] Compound **2** (1.82 g, 5.27 mmol) was dissolved in benzaldehyde (8.61 g, 81.16 mmol) and $ZnCl_2$ (2.82 g, 20.70 mmol) was added to the solution. The reaction mixture was stirred at room temperature for three days. The mixture was then diluted with ethyl acetate (200 ml) and washed with water (2× 300 ml). The combined water layers were washed with ethyl acetate (100 ml). The organic layers were pooled, dried with $MgSO_4$ and evaporated to dryness. The crude product was purified by silica gel column chromatography (stepwise gradient of 0–10% v/v MeOH in $CHCl_3$). The product was obtained as a mixture of *endo*/*exo* stereoisomers. Yield (1.70 g, 3.91 mmol) 74%. R_f = 0.3 (MeOH/$CHCl_3$, 1:19, v/v).

3.3. Synthesis of 2′,3′-*O*,*O*-benzylideneadenosine and 2′,3′-*O*,*O*-benzylidene-8-bromoadenosine 5′-hydrogenphosphonate triethylammonium salts (4a and b)

For this reaction the in situ pyrophosphonate method was used.[10] To a solution of **3a** or **b** (0.69 mmol) in

dry pyridine (2 ml) a prepared solution of phosphonic acid (282.9 mg, 3.45 mmol) and pivaloyl chloride (208 mg, 1.72 mmol) in pyridine (2 ml) was added. The reaction was followed by TLC ($CHCl_3$/MeOH, 8:2, v/v). The reaction was quenched after 4 h by addition of a mixture of triethylamine (1.92 ml, 13.8 mmol) in water (1 ml). The reaction mixture was evaporated to dryness and the residue was dissolved in THF and was passed through a filter. The THF solution was evaporated to dryness and purification was performed by silica gel chromatography (flash mode) using MeOH/$CHCl_3$ 1:19 v/v, containing 0.1% triethylamine. Products were obtained as a mixture of *endo*/*exo* stereoisomers. 2′,3′-*O*,*O*-Benzylideneadenosine 5′-hydrogenphosphonate triethylammonium salt (**4a**): yield 55%. 2′,3′-*O*,*O*-Benzylidene-8-bromoadenosine 5′-hydrogenphosphonate triethylammonium salt (**4b**): yield (335 mg, 0.56 mmol) 81%.

3.4. N^{α}-Benzyloxycarbonyl-L-isoleucinol (9)

N^{α}-Benzyloxycarbonyl-L-isoleucinol (**9**) was synthesized using published procedure.[14] To a solution of L-isoleucinol (200 mg, 1.71 mmol) in dried dioxane (1.70 ml), a solution of Na_2CO_3 (580.12 mg, 5.47 mmol) in water (2 ml) was added. Benzyl chloroformate (337.61 mg, 1.98 mmol) was added in five portions, while the mixture was stirred in an ice bath. The reaction was monitored by TLC ($CHCl_3$/MeOH, 19:1, v/v). After 1 h, the mixture was neutralised to pH 7 by KH_2PO_4 (pH 3) and extracted with H_2O (20 ml) and ethyl acetate (2× 30 ml). The organic layer was dried with $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel chromatography using methanol in chloroform (1:19, v/v) as eluent. Yield (430 mg, 1.71 mmol) $\geqslant$ 99%. $R_f = 0.7$ (MeOH/$CHCl_3$, 1:19, v/v).

3.5. N^{α}-Benzyloxycarbonyl-L-methioninol (10)

Compound **10** was prepared using L-methioninol as starting material following the above-described procedure for the synthesis of compound **9**. Yield (297 mg, 1.17 mmol) 94%. $R_f = 0.5$ (MeOH/$CHCl_3$, 1:19, v/v).

3.6. N^{α},N^{τ}-Dibenzyloxycarbonyl-L-histidinol (11)

N^{α},N^{τ}-Dibenzyloxycarbonyl-L-histidinol (**11**) was synthesized using published procedure.[14] To a solution of L-histidinol dihydrochloride (104 mg, 0.486 mmol) in dried dioxane (3 ml), a solution of Na_2CO_3 (483 mg, 4.55 mmol) in water (1.42 ml) was added. Benzyl chloroformate (182 mg, 1.07 mmol) was added in five portions, while the mixture was stirred in an ice bath. The reaction was followed by TLC ($CHCl_3$/MeOH, 19:1, v/v). After 1 h, the mixture was neutralised to pH 7 by KH_2PO_4 (pH 3) and extracted with H_2O (20 ml) and ethyl acetate (2× 30 ml). The organic layer was dried with $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel chromatography using methanol in chloroform (1:19, v/v) as eluent. Yield (82 mg, 0.20 mmol) 41%. $R_f = 0.8$ (MeOH/$CHCl_3$, 1:19, v/v).

3.7. Synthesis of protected 8-bromoadenosine and adenosine 5′-(aminoalkyl phosphate) triethylammonium salts (5a–d)

Compounds **4a** or **b** (0.25 mmol) and Z-amino alcohol (1.2 equiv, 0.30 mmol) were dried by evaporation of added pyridine (2× 3 ml) and then dissolved in dry pyridine (10 ml). The stirred reaction mixture was then treated with bis (2-oxo-oxazolin-3-yl) phosphonic chloride (OXP) (2 equiv, 0.5 mmol). The reaction was followed by TLC ($CHCl_3$/MeOH, 8:2, v/v) and after 30 min, iodine in a mixture of pyridine and water (2% w/v, 4.9 ml pyridine in 0.1 ml water) was added. The oxidation mixture was kept for 15 min, diluted with dichloromethane (10 ml) and evaporated to dryness. The residue was dissolved in dichloromethane (15 ml) and washed with a solution of $Na_2S_2O_3$ (10% aq) and triethylammonium bicarbonate (aq, 2 M) (1:1, v/v) (10 ml). The organic layer was dried with $MgSO_4$ and evaporated to dryness. The residue was purified by silica gel column chromatography (flash mode) using $CHCl_3$/MeOH (19:1) containing 0.1% triethylamine. Products were obtained as a mixture of *endo*/*exo* stereoisomers. 2′,3′-*O*,*O*-Benzylideneadenosine 5′-(N^{α}-benzyloxycarbonyl-L-isoleucinyl phosphate) triethylammonium salt (**5a**): yield 88%, $R_f = 0.2$ (MeOH/$CHCl_3$, 2:8, v/v). 2′,3′-*O*,*O*-Benzylidene-8-bromoadenosine 5′-(N^{α}-benzoxycarbonyl-L-isoleucinyl phosphate) triethylammonium salt (**5b**): yield 93%, $R_f = 0.5$ (MeOH/$CHCl_3$, 2:8, v/v). 2′,3′-*O*,*O*-Benzylidene-8-bromoadenosine 5′-(N^{α},N^{τ}-Dibenzyloxycarbonyl-L-histidinyl phosphate) triethylammonium salt (**5c**): yield 93%, $R_f = 0.4$ (MeOH/$CHCl_3$, 2:8, v/v). 2′,3′-*O*,*O*-Benzylidene-8-bromoadenosine 5′-(N^{α}-benzyloxycarbonyl-L-methioninyl phosphate) triethylammonium salt (**5d**): yield 86%, $R_f = 0.3$ (MeOH/$CHCl_3$, 2:8, v/v).

3.8. Synthesis of protected 8-azidoadenosine (aminoalkyl phosphate) triethylammonium salts (6b–d)

Compounds **5b**,**c** or **d** (0.23 mmol) was dissolved in DMSO (1 ml) and NaN_3 (4 equiv, 0.99 mmol) was added. The resulting solution was heated at 75 °C for 16 h, cooled to room temperature and diluted with THF (10 ml) whereupon excess NaN_3 largely precipitated. The mixture was passed through a filter and the solution was evaporated to dryness. The residue was purified by silica gel chromatography using $CHCl_3$/MeOH (19:1) containing 0.1% triethylamine. Products were obtained as a mixture of *endo*/*exo* stereoisomers. 2′,3′-*O*,*O*-Benzylidene-8-azidoadenosine 5′-(N^{α}-carbobenzoxy-L-isoleucinyl phosphate) triethylammonium salt (**6b**): yield 70%, $R_f = 0.7$ (THF/pyridine/H_2O, 8:1:1, v/v/v). 2′,3′-*O*,*O*-Benzylidene-8-azidoadenosine 5′-(N^{α},N^{τ}-dibenzyloxycarbonyl-L-histidinyl phosphate) triethylammonium salt (**6c**): yield 77%, $R_f = 0.7$ (THF/pyridine/H_2O, 8:1:1, v/v/v). 2′,3′-*O*,*O*-Benzylidene-8-azidoadenosine 5′-(N^{α}-benzyloxycarbonyl-L-methioninyl phosphate) triethylammonium salt (**6d**): yield 76%, $R_f = 0.8$ (THF/pyridine/H_2O, 8:1:1, v/v/v).

3.9. 8-Aminoadenosine and adenosine 5′-(L-isoleucinyl phosphate) triethylammonium salts (7a and 6a)

To palladium on carbon (100 mg), under a hydrogen atmosphere in 80% acetic acid (1 ml), was added **5a** or **6b** (27 mg, 0.033 mmol) in 80% acetic acid (2 ml). The reaction was stirred followed by TLC (2-propanol/ammonia/water, 7:1:2, v/v/v). After 24 h under hydrogen atmosphere, the mixture was diluted with water and methanol (50:50, v/v) (5 ml). The solution was filtered through a column of Celite, which was washed repeatedly with methanol, methanol–water and water. The combined solutions were evaporated to dryness under reduced pressure and the residue was redissolved in water and lyophilized. Reverse-phase HPLC purification was carried out using Jones (Genesis C18, 4μ, 10 × 250 mm) chromatography column employing a flow rate of 4 ml/min with a linear gradient 0–12.5% acetonitrile in 0.1 M triethylammonium acetate buffer (pH 6.5) at 30 °C for 90 min. The retention time of **6a** was 32 min and of **7a** 35 min. The purified product was lyophilized, redissolved in water and lyophilized again three times.

3.10. Adenosine 5′-(L-isoleucinyl phosphate) triethylammonium salt (6a)

Yield 82%. MS, (Q-TOF, *m*/*z*) calcd: $[M+H]^+$ = 447.17, found: $[M+H]^+$ = 447.07. R_f = 0.6 (2-propanol/ammonia/water, 7:1:2, v/v/v). ^{1}H NMR (400 MHz, D_2O) δ 0.74 (m, 6H, $2CH_3$), 0.82 (m, 1H, CH), 1.17 (m, 1H, CH_2A), 1.34 (t, $N(CH_2CH_3)_3$), 1.60 (m, 1H, CH_2B), 3.27 (q, $N(CH_2CH_3)_3$), 3.27 (1H, αCH under $N(CH_2CH_3)_3$), 3.81 (t, 2H, CH_2OP), 4.16 (m, 2H, 5′A/5′B), 4.42 (m, 1H, 4′), 4.62 (t, 1H, 3′), 4.92 (t, 1H, 2′), 6.16 (d, 1H, 1′), 8.27 (s, 1H, 2), 8.51 (s, 1H, 8) ppm. ^{13}C NMR (100 MHz, D_2O) δ 8.9, 10.9, 13.6, 25.4, 34.8, 47.3, 55.9, 56.0, 63.6, 63.6, 65.4, 65.4, 70.9, 74.1, 84.1, 84.2, 87.5, 119.1, 140.3, 149.8, 153.6, 156.2 ppm. ^{31}P NMR (162 MHz, D_2O) δ −0.3 ppm.

3.11. 8-Aminoadenosine 5′-(L-isoleucinyl phosphate) triethylammonium salt (7a)

Yield (14 mg, 0.024 mmol), 76%. Electrospray-MS: *m*/*z*, calcd: $[M+H]^+$ = 462.1866, found: $[M+H]^+$ = 462.1862, [M−Ile] = 363.0817. R_f = 0.5 (2-propanol/ammonia/water, 7:1:2, v/v/v). ^{1}H NMR, (400 MHz, D_2O/CD_3OD) δ 0.71 (m, 6H, $2CH_3$), 0.93 (m, 1H, CH), 1.21 (t, $N(CH_2CH_3)_3$), 1.21 (1H, CH_2A under $N(CH_2CH_3)_3$), 1.59 (m, 1H, CH_2B), 3.14 (q, $N(CH_2CH_3)_3$), 3.29 (m, 1H, αCH), 3.82 (m, 1H, CH_2OP), 3.93 (m, 1H, CH_2OP), 4.12 (m, 2H, 5′A/5′B), 4.23 (s, 1H, 4′), 4.44 (t, 1H, 3′), 4.86 (m, 1H, 2′), 5.91 (d, 1H, 1′), 7.97 (s, 1H, 2) ppm. ^{13}C NMR (100 MHz, D_2O/CD_3OD) δ 8.9, 10.9, 13.8, 25.5, 34.9, 47.3, 56.0, 56.0, 63.9, 64.0, 65.5, 65.5, 70.0, 71.2, 83.7, 83.8, 87.6, 116.9, 149.8, 150.2, 152.6, 153.0 ppm. ^{31}P NMR (162 MHz, D_2O/CD_3OD) δ −0.4 ppm.

3.12. 8-Aminoadenosine 5′-(L-histidinyl phosphate) triethylammonium salt (7b)

A solution of **6c** (33 mg, 0.034 mmol), in 80% acetic acid (2 ml) was added to a round-bottomed flask containing approximately 100 mg palladium black catalyst and 80% acetic acid (2 ml) with stirring under a hydrogen atmosphere. The progress of hydrogenation was followed by TLC (2-propanol/ammonia/water, 7:1:2, v/v/v) which indicated that the reaction was complete after 24 h. The crude compound was isolated by filtering off the catalyst through Celite and washing with methanol-water (50:50, v/v) (30 ml), followed by methanol (40 ml) and water (40 ml). The combined filtrates were then concentrated by evaporation under reduced pressure. The product was redissolved in water and lyophilized. Reverse-phase HPLC purification was carried out using Jones (Genesis C18, 4μ, 10 × 250 mm) chromatography column employing a flow rate of 4 ml/min with a linear gradient 0–12.5% acetonitrile in 0.1 M triethylammonium acetate buffer (pH 6.5) at 30 °C for 90 min. Retention time was 19.2 min. The purified product was lyophilized, redissolved in water and lyophilized again three times. Yield (15 mg, 0.025 mmol) 75%. Electrospray-MS: *m*/*z*, calcd: $[M+H]^+$ = 486.1615, found $[M+H]^+$ = 486.1611, [M−His] = 363.0818. R_f = 0.7 in 2-propanol/ammonia/water, 7:1:2, v/v/v. ^{1}H NMR (400 MHz, D_2O) δ 1.18 (t, $N(CH_2CH_3)_3$), 2.85 (d, 2H, βCH$_2$), 3.08 (q, $N(CH_2CH_3)_3$), 3.60 (m, 1H, αCH), 3.81 (m, 1H, CH_2O), 3.93 (m, 1H, CH_2O), 4.03 (m, 2H, 5′), 4.15 (m, 1H, 4′), 4.26 (dd, 1H, 3′), 4.64 (1H, 2′) 5.88 (d, 1H, 1′), 6.96 (s, 1H, CH(im)), 7.67 (s, 1H, CH(im)), 7.93 (s, 1H, 2) ppm. ^{13}C NMR (100 MHz, D_2O) δ 8.9, 26.7, 47.3, 51.8, 51.9, 65.0, 65.9, 70.3, 71.3, 83.9, 84.0, 87.6, 116.8, 117.3, 131.6, 136.4, 149.8, 150.1, 152.4, 152.9 ppm. ^{31}P NMR, (162 MHz, D_2O/CD_3OD/DMSO-d_6) δ −0.7 ppm.

3.13. 8-Aminoadenosine 5′-(L-methioninyl phosphate) triethylammonium salt (8)

A solution of **6d** (20 mg, 0.024 mmol), in 80% acetic acid (1 ml) was added to a round-bottomed flask containing approximately 50 mg palladium black catalyst and 80% acetic acid (2 ml) with stirring under a hydrogen atmosphere. After 36 h, 40 μl thiophenol was added and the mixture was stirred for 1 h. The crude compound was isolated by filtering off the catalyst through Celite and washing with methanol/water (50:50, v/v, 20 ml), followed by methanol (20 ml) and water (20 ml). The combined filtrates were then concentrated by evaporation under reduced pressure. The crude product was dissolved in 80% acetic acid (3 ml) and the reaction solution was stirred for 100 h. The crude product was evaporated to dryness, the residue was dissolved in water (25 ml) and washed with $CHCl_3$ (2× 10 ml). The aqueous layer was evaporated to dryness, redissolved in water and lyophilized.

The crude product was dissolved in water (1 ml) and an excess of sodium hydrosulfite (dithionite) (10 mg, 0.06 mmol) was added. The solution was heated up to 60 °C and the reaction was followed by TLC (2-propa-

nol/ammonia/water, 7/1/2, v/v/v). After 36 h, the reaction mixture was diluted with a triethylammonium acetate buffer (pH 6.5) (2 ml). Reverse-phase HPLC purification was carried out using a Thermo Hypersil–Keystone (C18, 5μ, 10× 250 mm) column employing a flow rate of 3 ml/min with a linear gradient of 0–14% acetonitrile in 0.05 M triethylammonium acetate buffer (pH 6.5) at 50 °C for 30 min. The retention time was 23.2 min. The purified product was lyophilized, redissolved in water and lyophilized again three times. Yield (3.2 mg, 7 μmol), 35%. Electrospray-MS: *m*/*z*, calcd: $[M]^-$ = 478.1274, found: $[M]^-$ = 478.1360, R_f = 0.4 (2-propanol/ammonia/water, 7:1:2, v/v/v). ^{1}H NMR (400 MHz, D_2O/CD_3OH) δ 1.33 (t, $N(CH_2C\underline{H}_3)_3$), 1.91 (q, 2H, βCH_2), 2.04 (s, 3H, SCH_3), 2.54 (t, 2H, CH_2S), 3.25 (q, $N(CH_2CH_3)_3$), 3.62 (m, 1H, αCH), 3.95 (m, 1H, CH_2OP), 4.10 (m, 1H, CH_2OP), 4.25 (m, 2H, 5′), 4.36 (m, 1H, 4′), 4.54 (dd, 1H, 3′), 4.92 (t, 1H, 2′), 6.06 (d, 1H, 1′), 8.12 (s, 1H, 2) ppm. ^{13}C NMR (100 MHz, D_2O/CD_3OD) δ 8.9, 14.5, 28.4, 29.3, 47.3, 51.0, 51.1, 65.3, 65.6, 70.1, 71.3, 83.9, 84.0, 87.6, 117.0, 150.0, 150.3, 152.7, 153.0 ppm. ^{31}P NMR (162 MHz, D_2O/CD_3OD) δ −0.4 ppm.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.11.049.

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