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A Practical Route to 3′-Amino-3′-deoxyadenosine Derivatives and Puromycin Analogues

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Abstract: 3′-Aminoacylamino-3′-deoxyadenosines, analogues of the antibiotic puromycin, have been synthesized from adenosine. They key 3′-azido derivative **10** was obtained through a 3′-oxidation/reduction/substitution procedure. A modified purification protocol on a larger scale was developed for the oxidation step using the Garegg reagent. The coupling reaction between an Fmoc-L-amino acid and the fully protected form of 3′-amino-3′-deoxyadenosine **11** furnished the aminoacylated compounds **12** in high yields. The puromycin analogues were obtained in 10 steps and up to 23% (**14c**) overall yield.

The antibiotic puromycin (**1**, Figure 1), a metabolite of *Streptomyces alboniger*, was first isolated by Porter and co-workers in 1952.[1] Puromycin has been and is being extensively used for the elucidation of the mechanism of the protein biosynthesis.[2] Yarmolinsky and De la Haba[3] were the first to recognize close structural similarity between puromycin and the aminoacyl end of aminoacyl-tRNA. Further studies[4−7] showed that puromycin inhibits protein synthesis by transferring the ribosomal nascent peptide to the puromycin α-amino group. Five years ago, a novel application of puromycin was introduced in the laboratories of Yanagawa and of Roberts and Szostak.[8] The principle, based on the peptidyl transfer ability of puromycin, allows for the in vitro selection of proteins or RNA strands via mRNA-peptide fusions using puromycin as the point of covalent attachment. A number of synthetic routes to puromycin and analogues have been reported,[9] but most of them suffer from numerous steps and a low overall yield. In 1989, Samano and Robins[10] were the first to publish an efficient synthetic pathway to puromycin. The latter recently reported details[11] of their nine-step synthesis to 3′-aminodeoxynucleoside **2** with an improved but, in our hands, still difficult procedure for the problematic final N-debenzylation reaction. We communicated our synthesis of 3′-alanylamino-3′-deoxyadenosine from adenosine in 10 steps[12] and now report the optimized protocol for a general synthesis of puromycin analogues **14**.

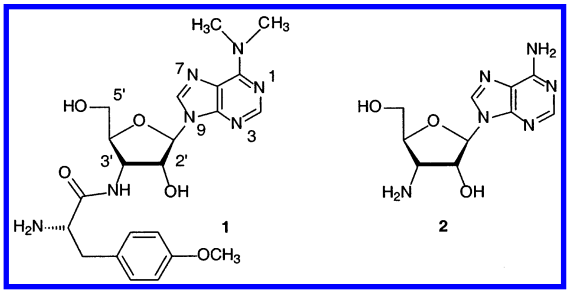

FIGURE 1. Puromycin (**1**) and 3′-amino-3′-deoxyadenosine (**2**).

Our synthetic pathway utilizes a protected form of 3′-amino-3′-deoxyadenosine (**2**) synthesized from adenosine. The 2′,5′-bis-*O*-TBDMS xylofuranosyladenine derivative **7** (Scheme 1) was synthesized in three steps as described in the literature.[13] Thus, the silylation furnished at most 60% of the desired 2′,5′-bis-*O*-silylated isomer **3**. The other products were 3′,5′-bis-*O*-silylated adenosine **4** (34%) and 2′,3′,5′-tris-*O*-silylated adenosine **5** (traces); no monosilylated product was isolated. To improve the yield of **3**, we isomerized compound **4** in a solution of 2.5% (v/v) triethylamine in methanol, so **3** was obtained in 85% yield after two isomerizations. The oxidation of the 3′-hydroxyl group was achieved using the Garegg reagent.[13c] The isolation of the ketone **6** on a >3 g scale, by

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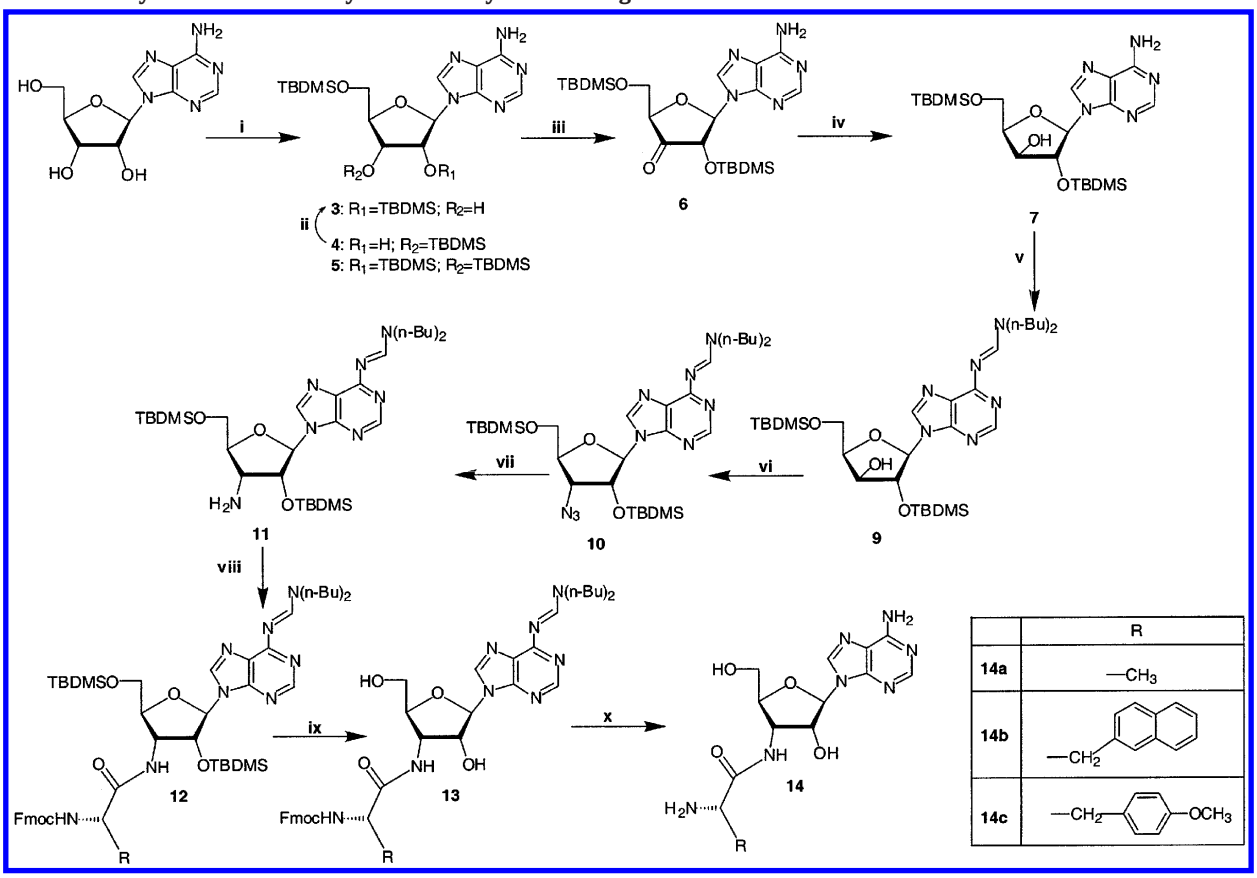
SCHEME 1. Synthetic Pathway to Puromycin Analogues[a]



[a] Key: (i) TBDMS-Cl, pyridine, rt; (ii) 2.5% (v/v) Et_3N in MeOH, rt, 85%; (iii) CrO_3, Ac_2O, pyridine, rt, 85-91%; (iv) $NaBH_4$, AcOH, 8 °C, 84%; (v) **8** [= $(CH_3O)_2HC$-N(*n*-$Bu)_2$], MeOH, rt, 97%; (vi) (1) 2 equiv of TfCl, DMAP, CH_2Cl_2, rt, (2) LiN_3, DMF, rt, 86%; (vii) (1) PPh_3, dioxane, rt, (2) H_2O, rt, 84%; (viii) Fmoc-L-amino acid, CBMIT,[18] CH_3NO_2, *N*-methylimidazole, THF, rt, **12a** (95%), **12b** (84%), **12c** (96%); (ix) pyridine−HF complex, THF, rt, **13a** (75%), **13b** (93%), **13c** (98%); (x) 40% piperidine in MeOH (v/v), rt, **14a**, **14b**, **14c** (54%).

precipitation with EtOAc as described in the literature,[13d] resulted in low yields because part of the product was trapped into the chromium−complex precipitate.

We therefore propose another purification procedure according to which the crude material is directly loaded onto a silica column and slowly eluted with CH_2Cl_2 to separate the product from the chromium complex. Then a step gradient elution with EtOAc/hexane (1:1−1:0) allows for the isolation of ketone **6**. Under our conditions, the product is already separated from the chromium complex and will not be trapped in the precipitate. This procedure increases the yield of the oxidation reaction, as we obtained pure, colorless, chromium-free, and storable **6** in 85−91% yield. The reduction of **6** (no traces of **3**) was performed at 12−13 °C using a cryostat. The xylo derivative **7** was isolated in 84% yield which contained approximatively 3% of the ribo isomer **3** as determined by ^1H NMR. This mixture was not further purified, and the contaminant was eliminated during the purification of the next steps. The introduction of the azido function into the 3′-position was performed through a nucleophilic substitution of the triflate-activated 3′-*xylo*-hydroxyl group with lithium azide.[14] We first attempted these reactions without protection of the 6-amino group of the adenine moiety. The best yield we obtained was only 50%; in fact, during the activation of the 3′-hydroxyl group with trifluoromethanesulfonyl chloride, the amino group of **7** was derivatized as well. To protect the amino group, we chose a base labile formamidine protecting group[15] using *N*,*N*-di(*n*-butyl)formamide dimethyl acetal (**8**), prepared as described by Froehler and Matteucci.[16] Compound **8** reacts with **7** within 2 h at room temperature to furnish **9** in 97% yield. Derivative **10** was isolated in 86% yield. The 3′-epimer of **10** that could have arisen from small amounts of contaminating **3** was absent in **9**, as shown by NMR and in comparison with intentionally synthesized material (same reaction sequence starting from **3** instead of **7**). The azido functionality was reduced to the amine **11** (84%) using the Staudinger reaction.[17] Amine **11** should not be stored for too long as an oil, since it contains a base-labile protection that is susceptible to slow cleavage through nucleophilic attack by the alkylamino function.

The key step in the reaction sequence is the coupling of an N-protected L-amino acid to the sterically hindered 3′-amino group of **11**. Saha, Schultz, and Rapoport reported on a coupling method in which, via a highly

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reactive acyl imidazolium intermediate, even secondary amines are coupled in high yields without an additional base and without racemisation of the carboxy component.[18]

We synthesized three analogues **12** (Scheme 1) bearing alanine, 3-(β-naphthyl)alanine, and *p*-methoxyphenylalanine (*O*-methyltyrosine) in isolated yields of, respectively, 95, 84, and 96% with respect to **11**. The 5′- and 2′-*O*-silyl groups were deprotected with the pyridine−HF complex reagent in 75−98% yields, and the deprotection of the two amino groups was performed with a solution of 40% piperidine in methanol. The analogues were submitted directly to purification by preparative reversed-phase HPLC furnishing target molecules **14a**, **14b**, and **14c**.

In conclusion, we describe here an efficient and reliable route to puromycin analogues in 10 steps. Under optimized and routine conditions, the protected compounds **13a**,**b**,**c** are isolated in 30−43% yields in nine steps starting from adenosine. Usually, we prepared target compounds **14a**,**b**,**c** on a 30 μmol scale without determining the yield of the final step; however, once we prepared **14c** from **13c** in 54% yield (excluding residual NH_4OAc) after RP-HPLC purification and repeated lyophilization, i.e., in 23% overall yield with respect to adenosine. Our synthetic pathway allows for a wide variety of amino acid side chains to be introduced. Although, for in vivo experiments, the 6-dimethylamino group is essential to escape the activity of adenosine deaminases, it is not needed for in vitro experiments on ribosomes as is the focus of this work. Several studies[19] have shown that puromycin's dimethylamino group is not necessary for its biological activity. Experiments on the kinetics of the ribosomal peptidyl transfer reaction using our puromycin analogues are currently underway.

Experimental Section

^{1}H NMR (300 and 400 MHz) and ^{13}C NMR (75 and 100 MHz) spectra were obtained with solutions in $CDCl_3$ using tetramethylsilane as internal standard. ^{1}H NMR spectra of the analogues **14** were obtained from solutions in H_2O/5% D_2O at 600 MHz using sodium 3-(trimethylsilyl)propionate as internal standard. The signal listings of all ^{1}H and ^{13}C NMR spectra are available in the Supporting Information. Mass spectra (MS and HRMS) were obtained using fast atom bombardement (*p*-nitrobenzyl alcohol) and chemical ionization (isobutane) methods and electrospray ionization (ESI in MeOH for **12** and **13** and H_2O/MeOH 9:1 for the analogues **14**). The melting points were measured on a Kofler block and are uncorrected. TLC was run on precoated silica gel F_{254} plates with fluorescent indicator. Nucleosides were visualized on TLC plates by subsequent spraying with concentrated H_2SO_4 and 2% naphthoresorcin solutions in ethanol, followed by heating. Column chromatography was performed with flash silica gel 60 (40−63 μm). Reagent grade chemicals were used. LiN_3 was prepared from NaN_3 and LiCl (1:1) in EtOH (residual NaN_3 and LiCl were filtered off after treatment with EtOH and then Et_2O). ***Caution:*** *do not acidify; do not heat as a dry solid!* Solvents were distilled before use for extractions. Tetrahydrofuran was dried by distillation from sodium/benzophenone. All other solvents were used as purchased. Prep HPLC: 250 × 8 mm Eurospher 100/5 RP_{18} column (Knauer), flow rate: 2 mL/min; UV detection at 260 nm. Isocratic conditions were used for the purification of the analogues **14** (77% 0.65 mM aq NH_4OAc/23% HPLC-grade CH_3CN).

2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-adenosine (3). Compound **3** was prepared as described in the ref 15c with the following reagents: adenosine (1 g, 3.74 mmol), TBDMS-Cl (1.7 g, 11.22 mmol), pyridine (8 mL). **Isomerization of 4.** To a suspension of a mixture of **3**/**4** with a high content of **4** (7.22 g, 14.5 mmol) in MeOH (90 mL) was added triethylamine (2.3 mL, 2.5% v/v), and the solution was stirred at rt for 2 h. The solution was evaporated to dryness, and purification by chromatography (EtOAc/hexane 7:3) furnished more pure **3** as a white solid. After two isomerizations, the yield of **3** for the silylation reaction increased to 85%: R_f **3**/**4** = 0.25/0.15 (EtOAc/hexane 7:3). Mp = 177−178 °C. MS (FAB) *m*/*z*: 496 [(M + H)$^+$, 100]. HRMS (CI): *m*/*z* (calcd [M + H]$^+$) 496.27753, *m*/*z* (found) 496.27778. Anal. Calcd for $C_{22}H_{41}N_5O_4Si_2$: C, 53.30; H, 8.34; N, 14.13. Found: C, 53.58; H, 8.37; N, 13.93.

9-(2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-*erythro*-pentofuran-3-ulosyl)-9*H*-adenine (6). Compound **6** was prepared as described in ref 13d with the following reagents: chromium(VI) oxide (CrO_3, 3.24 g, 32.4 mmol), acetic anhydride (3.0 mL, 32.4 mmol), pyridine (5.2 mL, 64.8 mmol), **3** (5.35 g, 10.8 mmol), and CH_2Cl_2 (57 mL). The purification procedure was modified as follows: the reaction mixture was directly poured onto the chromatography column (800 g flash silica conditioned with CH_2Cl_2 in a 8 cm ∅ column) and slowly eluted with CH_2Cl_2 until a yellow band reached the bottom of the column. Then a step gradient elution from EtOAc/hexane 1:1 to 1:0 afforded **6** (4.95 g, 91%) as a white solid. R_f = 0.46 (EtOAc). Mp = 175−177 °C. MS (FAB) *m*/*z*: 494 [(M + H)$^+$, 100]. HRMS (CI): *m*/*z* (calcd [M + H]$^+$) 494.26190, *m*/*z* (found) 494.26225. Anal. Calcd for $C_{22}H_{39}N_5O_4Si_2$: C, 53.52; H, 7.96; N, 14.18. Found: C, 53.36; H, 7.78; N, 14.24.

9-(2′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranosyl)-9*H*-adenine (7). Compound **7** was prepared as described in ref 13e with the following reagents: sodium borohydride (1 g, 39 mmol), acetic acid (48 mL), **6** (3 g, 6 mmol). A mechanical stirrer was used throughout the synthesis. During the addition of $NaBH_4$ to acetic acid, the internal temperature was kept at 15−16 °C. During the addition and reaction of **6**, the external cooling bath temperature was kept close to 8 °C resulting in an internal reaction temperature of 12−13 °C. After 2.5 days, the acetic acid was evaporated under reduced pressure and the yellow residue was taken up and extracted in an ice-cold mixture of saturated $NaHCO_3$ solution/EtOAc (1:3). The organic layer was washed with brine, dried over anhyd Na_2SO_4, and evaporated. Compound **7** (2.5 g, 84%) was obtained as a colorless foam. R_f = 0.38 (EtOAc). Mp = 65−69 °C. MS (FAB) *m*/*z* 496 [(M + H)$^+$, 100]. HRMS (CI): *m*/*z* (calcd [M + H]$^+$) 496.27753, *m*/*z* (found) 496.27767. Anal. Calcd for $C_{22}H_{41}N_5O_4Si_2$: C, 53.30; H, 8.34; N, 14.13. Found: C, 53.15; H, 8.11; N, 14.03.

6-*N*-[(Di-*n*-butylamino)methylene]-9-(2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-xylofuranosyl)-9*H*-adenine (9). Compound **7** (1.48 g, 2.98 mmol) was coevaporated three times with absolute pyridine under reduced pressure and dissolved in 10 mL of methanol. After addition of *N*,*N*-di-*n*-butylformamide dimethyl acetal[16] (8, 1.21 g, 5.96 mmol), the solution was stirred at rt for 2 h followed by evaporation. Purification by chromatography (EtOAc/hexane 1:1) furnished **9** (1.84 g, 97%) as a yellowish oil. R_f = 0.55 (EtOAc/hexane 1:1). MS (FAB) *m*/*z*: 636 [(M + H)$^+$, 100].

3′-Azido-6-*N*-[(di-*n*-butylamino)methylene]-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-β-D-adenosine (10). Compound **9** (2.00 g, 3.15 mmol) and DMAP (0.96 g, 7.87 mmol) were coevaporated with anhydrous CH_2Cl_2 under reduced pressure and then dissolved in anhydrous CH_2Cl_2 (8 mL). The solution was cooled to 0 °C, and trifluoromethylsulfonyl chloride (0.67 mL, 6.39 mmol) was added dropwise. After being stirred at 0 °C for 30 min, the yellow solution was taken up in AcOH/H_2O (1:99)/CH_2Cl_2 and extracted twice. The organic layers were washed with ice-cold saturated $NaHCO_3$ solution and brine, dried over anhyd Na_2SO_4, evaporated, and dried under high vacuum (oil pump) resulting in a yellow oil. The residue was taken up in DMF (30 mL) and added to solid lithium azide (LiN_3,

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0.77 g, 15.75 mmol) under argon. The solution was stirred at rt overnight and then taken up in saturated $NaHCO_3$ solution/EtOAc. After extraction, the organic layers were combined and washed with brine, dried over anhyd Na_2SO_4, and evaporated. Purification by chromatography (EtOAc/hexane 1:1) gave **10** (1.79 g, 86%) as a colorless oil. $R_f = 0.54$ (EtOAc/hexane 1:1). IR (KBr): 2107.5 cm^{-1} (N_3 stretch). MS (FAB) *m/z*: 660 [$(M + H)^+$, 100]. HRMS (FAB): (M + H) *m/z* (calcd) 660.42012; *m/z* (found) 660.42016.

3′-Amino-6-*N*-[(di-*n*-butylamino)methylene]-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-deoxy-*β*-D-adenosine (11). To a solution of **10** (483 mg, 0.732 mmol) in dioxane (14.5 mL, filtered through Al_2O_3 to remove traces of peroxide) was added PPh_3 (250 mg, 0.952 mmol), and the solution was stirred at rt overnight. H_2O was added (9 mL), and the solution was stirred for 2 h at rt. The solution was taken up in saturated $NaHCO_3$ solution/EtOAc and extracted twice. The organic layers were combined, washed with brine, dried over anhyd Na_2SO_4, and evaporated. After chromatographic purification (EtOAc), **11** (390 mg, 84%) was obtained as a colorless oil. $R_f = 0.36$ (EtOAc). IR (KBr): 3678/3624 cm^{-1} (NH_2 stretch). MS (FAB) *m/z*: 634 [$(M + H)^+$, 100]. HRMS (FAB): (M + H) *m/z* (calcd) 634.42962, *m/z* (found) 634.42919.

6-*N*-[(Di-*n*-butylamino)methylene]-2′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-3′-[*N*-(9-fluorenyl)methoxycarbonyl-L-aminoacyl]-3′-deoxy-*β*-D-adenosine (12). To a solution of carbonyl diimidazole (38.4 mg, 0.24 mmol) in absolute CH_3NO_2 (0.45 mL) at 0 °C was added methyl triflate (51.8 μL, 0.48 mmol), and the solution was stirred for 10 min while warming to rt. The solution was added to a suspension of *N*-(9-fluorenyl)-methoxycarbonyl-L-amino acid (0.24 mmol) and *N*-methylimidazole (1.2 μL, 0.016 mmol) in absolute CH_3NO_2 (0.6 mL) resulting in a clear solution that was stirred at rt until the production of CO_2 had ceased. Then a solution of **11** (50 mg, 0.08 mmol) in THF (1 mL) was added under argon. After being stirred at rt for 2 h, the solution was taken up in a CH_2Cl_2/H_2O and extracted three times. The organic layers were combined, washed with saturated $NaHCO_3$ solution and H_2O, dried over anhyd Na_2SO_4, and evaporated. After chromatographic purification (EtOAc), compounds **12** were obtained as oils.

12a ("Ala"). $R_f = 0.35$ (EtOAc/hexane 7:3); 70.5 mg, 95%. MS (ESI) *m/z*: 928 [$(M + H)^+$, 100].

12b ("Napht"). $R_f = 0.31$ (EtOAc/hexane 1:1); 70.8 mg, 84%. MS (ESI) *m/z*: 1054 [$(M + H)^+$, 100].

12c ("*O*-MeTyr"). $R_f = 0.51$ (EtOAc/hexane 1:1); 79.3 mg, 96%. (Largest scale: 700 mg **12c** = 96% isolated yield.) MS (ESI) *m/z*: 1034 [$(M + H)^+$, 100].

6-*N*-[(Di-*n*-butylamino)methylene]-3′-[*N*-(9-fluorenyl)-methoxycarbonyl-L-aminoacyl]-3′-deoxy-*β*-D-adenosine (13). To a solution of **12** (0.05 mmol) in THF (0.5 mL) in an Eppendorf tube was added pyridine−hydrogen fluoride (∼70% HF) (11 μL, 0.12 mmol). The solution was stirred at rt for 4 h and then taken up in saturated $NaHCO_3$ solution/EtOAc and extracted three times. The organic layer was washed with brine, dried over anhyd Na_2SO_4, and evaporated. Purification by chromatography (step gradient from EtOAc to EtOAc/MeOH 9:1) furnished **13** as an oil.

13a ("Ala"). $R_f = 0.42$ (EtOAc/MeOH 9:1); 26.3 mg, 75%. MS (ESI) *m/z*: 700 [$(M + H)^+$, 100].

13b ("Napht"). $R_f = 0.66$ (EtOAc/MeOH 9:1); 38.4 mg, 93%. MS (ESI) *m/z*: 826 [$(M + H)^+$, 100].

13c ("*O*-MeTyr"). $R_f = 0.55$ (EtOAc/MeOH 9:1); 39.4 mg, 98%. MS (ESI) *m/z*: 794 [$(M + H)^+$, 100].

3′-L-Aminoacylamino-3′-deoxy-*β*-D-adenosine (14). To **13** (0.03 mmol) was added a solution (0.59 mL) of 40% (v/v) piperidine in MeOH. The mixture was stirred at rt for 2 h, evaporated to dryness, and directly submitted to purification by RP-HPLC. The fractions containing **14** were concentrated in a $Si(CH_3)_2Cl_2$-treated glass flask on a rota-evaporator under reduced pressure and then repeatedly lyophilized in Eppendorf tubes under high-vacuum until NH_4OAc was present in lower than 5 molar equiv amounts with respect to the analogues **14**, as determined by ^1H NMR. The analogues **14** were thus isolated as white fluffy solids.

3′-L-Alanylamino-3′-deoxy-*β*-D-adenosine (14a). t_R (260 nm) = 12−13 min. MS (ESI) *m/z*: 338 [$(M + H)^+$, 100].

3′-L-(3-(*β*-Naphthyl)alanylamino)-3′-deoxy-*β*-D-adenosine (14b). t_R (260 nm) = 24−25 min. MS (ESI) *m/z*: 464 [$(M + H)^+$, 100].

3′-L-(*p*-Methoxyphenylalanylamino)-3′-deoxy-*β*-D-adenosine (14c). t_R (260 nm) = 14−15 min (1.5 mg **14c**·2.3NH_4OAc = isolated yield 54%). MS (ESI) *m/z*: 444 [$(M + H)^+$, 100].

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Supporting Information Available: ^1H NMR spectra, ^1H and ^{13}C NMR signal listings of the compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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