

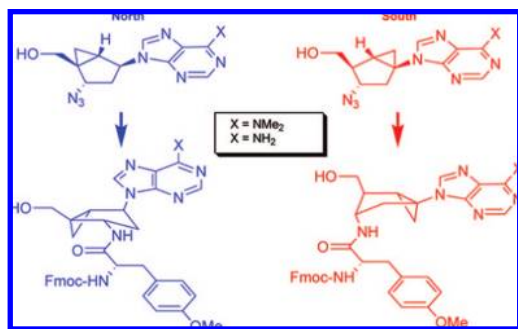
Synthesis of Conformationally Locked Versions of Puromycin Analogues

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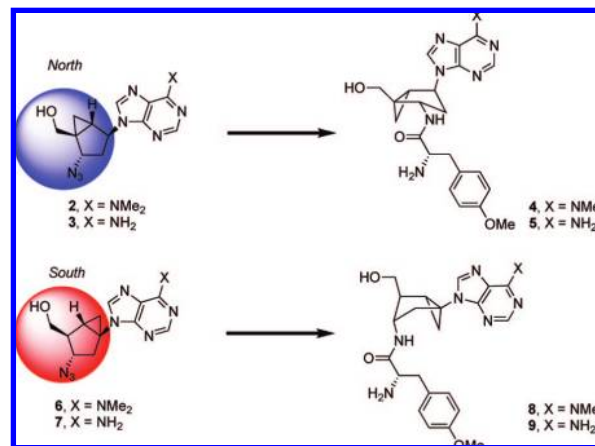
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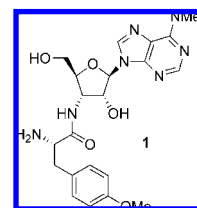
Conformationally locked *North* and *South* versions of puromycin analogues built on a bicyclo[3.1.0]hexane pseudosugar template were synthesized. The final assembly of the products was accomplished by the Staudinger–Vilarrasa coupling of the corresponding *North* (**2** and **3**) and *South* (**6** and **7**) 3'-azidopurine carbanucleosides with the Fmoc-protected 1-hydroxybenzotriazole ester of 4-methoxy-L-tyrosine. *North* azides **2** and **3** were reported earlier. The 3'-azido intermediates **6** and **7** that are necessary for the synthesis of the *South* puromycin analogues are described herein for the first time.

Puromycin (**1**) is a 3'-amino-3'-deoxynucleoside antibiotic produced by *Streptomyces alboniger*¹ that specifically inhibits peptidyl transfer on both prokaryotic and eukaryotic ribosomes by interfering with RNA function and leading to premature chain termination during translation.^{2,3} Like the natural substrate, but

SCHEME 1



independent of the codon, it enters the A site because of its molecular resemblance to the 3' end of the normal ester-linked 3'-aminoacyl tRNA. Its primary amino group is capable of taking over the nascent peptide chain; however, the resulting more stable amide bond is resistant to further nucleophilic attack. This causes the ribosome to stop peptide chain elongation and to release a truncated C-terminal puromycyl peptide, which may be lethal to the organism.



Because of the important role of sugar pucker in nucleosides, we have proposed the use of conformationally locked carbocyclic nucleosides built on a rigid bicyclo[3.1.0]hexane scaffold to restrict the embedded cyclopentane pseudosugar ring to either a *North*-type (₂E) or a *South*-type (₃E) conformation as defined in the pseudorotational cycle.⁴ These types of modified nucleoside analogues have been used to study the role of sugar conformation in the processes of recognition and binding of nucleosides, nucleotides, and oligonucleotides to their target enzymes.⁵

In the present work, we have expanded the scope of our investigations in this area by synthesizing the puromycin analogues in both *North*-type (**4**) and *South*-type flavors (**8**), plus analogues **5** and **9** containing the 6-aminopurine base (Scheme 1). The latter compounds are also valid probes because

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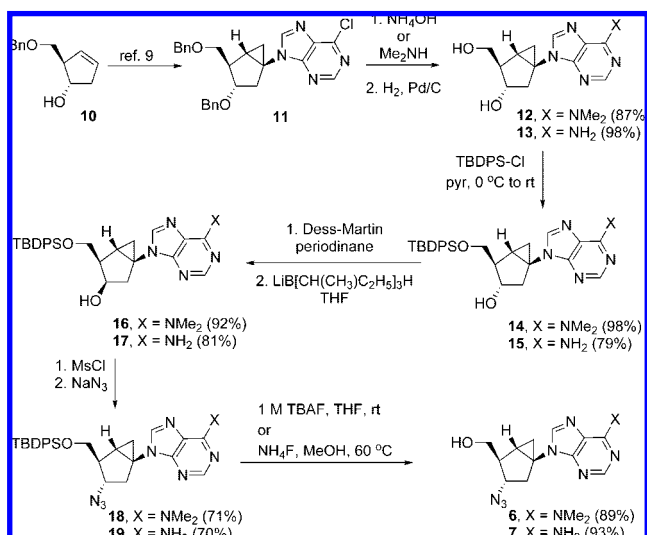
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SCHEME 2



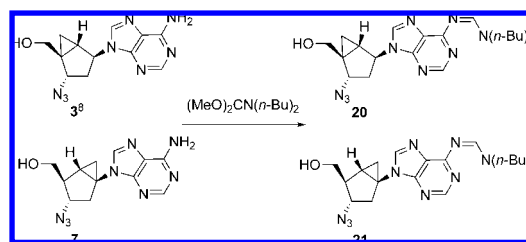
the methyl substituents on the dimethylamino group are not essential for puromycin-like activity.⁶

These novel puromycin ensembles (Scheme 1) should help determine the preferred conformation of the 3'-terminal ribosyl residue of peptide-accepting aminoacyl-tRNA at the critical A site. Although the proposed target compounds lack the 2'-OH, the bicyclo[3.1.0]hexane ring system is able to precisely confine the conformation of the pseudosugar regardless of the presence of the 2'-OH. In 1990 Koizumi et al. synthesized 2'-deoxy-puromycin and suggested that the weaker antimicrobial properties of the compound were due to a conformational dominance of the *South* conformation in contrast to puromycin's *North* conformation that was revealed by proton NMR.⁷ Hence, the target compounds (**4** and **5**, **8** and **9**) are proposed to reliably test whether the puckering of the sugar ring changes the orientation of the aminoacyl moiety of puromycin and how it influences the recognition by peptidyl transferase at the active site.

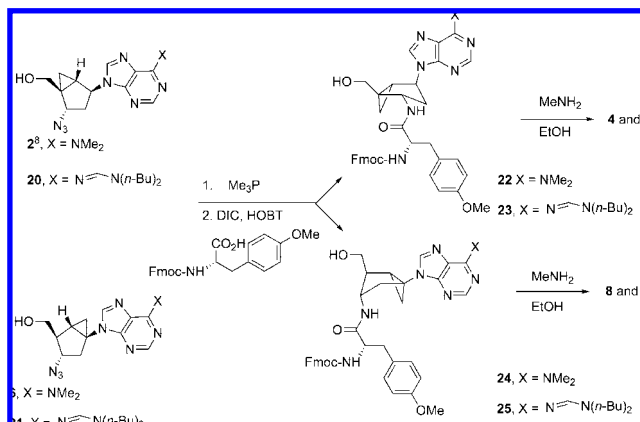
Compounds **2** and **3** in the *North* hemisphere, which are necessary for the synthesis of **4** and **5**, have already been made and reported.⁸ For the *South* puromycin analogues a similar strategy required the synthesis of the critical 3'-azido intermediates **6** and **7** as shown in Scheme 2.

Starting from known cyclopentanol **10**, the synthesis of **11** was performed in 12 steps (4.2% yield) as previously described.⁹ Conversion of the 6-chloro derivative to either the 6-dimethylamino- or 6-aminopurine carbobicyclic nucleosides **12** and **13** was easily accomplished after treatment with dimethylamine or ammonium hydroxide, respectively. After protection of the primary alcohol as silyl ethers **14** and **15**, the strategy to invert the configuration of the 3'-OH (nucleoside numbering) was performed by a tandem oxidation–reduction methodology using Dess–Martin oxidation followed by reduction with L-selectride to give **16** and **17**. This process was more efficient than the inversion of configuration approach reported earlier for the *North*

SCHEME 3



SCHEME 4



series using Mitsunobu reaction conditions.⁸ Formation of the mesylate esters and a second inversion of configuration resulting from the nucleophilic attack with sodium azide produced compounds **18** and **19**, which after the removal of the silyl ether provided the key target 3'-azides **6** and **7** in satisfactory yields.

In order to make the corresponding 6-aminopurine puromycin analogues, the amino group required protection, which was accomplished by reacting with *N,N*-di(*n*-butyl)formamide dimethyl acetal prepared as described by Froehler and Matteucci¹⁰ and reacted with *North* and *South* 6-aminopurine analogues, **3**⁸ and **7** to give the 6-*N*-[(di-*n*-butylamino)methylene]-protected compounds **20** and **21** (Scheme 3).

Having the necessary 3'-azide precursors at hand, we then employed the very efficient, one-step Villarrasa–modified¹¹ Staudinger coupling utilizing the activated amino acid, 4-methoxy-L-tyrosine, protected as the *N*-(9-fluorenylmethyl)-carbamate (*N*-Fmoc-OMe-L-Tyr) as shown in Scheme 4. Previously, our group had successfully employed and optimized this approach for the synthesis of a number of noncarbocyclic puromycin analogues.^{12–15} Thus, a mixture of *N*-Fmoc-OMe-L-Tyr and anhydrous 1-hydroxybenzotriazole (HOBT) was treated with 1,3-diisopropylcarbodiimide (DIC) and then chemoselectively added to the phosphine imines that were generated from the reaction between the azides and trimethylphosphine. The final coupling yields after deprotection were ca. 78%.

In summary, we have achieved the syntheses of conformationally locked *North* and *South* versions of puromycin ana-

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logues on a bicyclo[3.1.0]hexane pseudosugar template. These compounds were designed to investigate the ideal conformation of the 3'-terminal ribofuranose ring of 3'-aminoacyl tRNAs during takeover of the nascent peptide in the ribosome. The biological evaluation of these compounds will be reported elsewhere.

Experimental Section

(2S)-N-[(1R,2S,4S,5S)-4-[6-(Dimethylamino)purin-9-yl]-1-(hydroxymethyl)bicyclo[3.1.0]hex-2-yl]-2-[(fluoren-9-ylmethoxy)-carbonylamino]-3-(4-methoxyphenyl)propanamide (22). A mixture of *N*-Fmoc-*O*-Me-L-Tyr (22 mg, 0.063 mmol) and HOBt (8.6 mg, 0.053 mmol) was coevaporated three times from anhydrous THF (3 mL). The residue was dissolved in THF (1.0 mL), and the solution was cooled down to 0 °C under N₂ for 10 min. Then, DIC (34 μL, 0.212 mmol) was added, and the reaction mixture was stirred for 10 min at the same temperature. Nearby, (Me)₃P (1 M in THF, 80 μL, 0.080 mmol) was added to a solution of the azide **2**⁸ (12 mg, 0.038 mmol) in THF (1.0 mL), which was stirred for 1 min at room temperature. The amino acid solution was warmed to room temperature during 1 min and then added to the iminophosphorane solution. The reaction mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure, coevaporated from CHCl₃ (5 mL), then dissolved in EtOAc (15 mL), and extracted with saturated NaHCO₃ (8 mL). The organic layer was extracted twice with EtOAc, washed with H₂O (2 × 5 mL), dried (MgSO₄), filtered, and evaporated under reduced pressure. The oily residue was purified by silica gel column chromatography (EtOAc/cyclohexane/MeOH, 2/1/0 → 3/1/0 → 4/1/0 → 5/1/0 → 4/1/0.7, v/v) to give the coupling product **22** (23 mg, 89%) as a colorless resin.

(2S)-N-[(1R,2S,4S,5S)-4-[6-(Dimethylamino)purin-9-yl]-1-(hydroxymethyl)bicyclo[3.1.0]hex-2-yl]-2-amino-3-(4-methoxyphenyl)propanamide (4). Compound **22** (20 mg, 0.071 mmol) was dissolved in 33% CH₃NH₂/EtOH (3 mL). The reaction mixture was stirred at room temperature overnight in a closed vessel. The solution was concentrated in vacuo and coevaporated from CHCl₃ (5 mL). The oily residue was purified by silica gel column chromatography (EtOAc/toluene/MeOH, 5/1/0.5 → 4/1/0.5, v/v; EtOAc/MeOH/H₂O, 8/1/0.5 → 6/1/0.5 → 4/1/0.5, v/v) to yield after evaporation the target compound **4** (13.5 mg, 99%) and, after lyophilisation from H₂O/TFA (pH 3.0), the more water-soluble salt **4**·TFA as a hygroscopic solid: ¹H NMR (CD₃OD, 300 MHz) δ 0.73 (1H, dd, *J* = 6.0, 3.9 Hz, H-6'a), 0.90 (1H, dd, *J* = 6.0, 3.9 Hz, H-6'b), 1.68–1.79 (2H, m, H-3'a, H-5'), 2.07 (1H, dd, *J* = 14.7, 8.1 Hz, H-3'b), 2.87 (1H, dd, *J* = 13.6, 6.8 Hz, *p*-MeOPhCHH), 2.95 (1H, dd, *J* = 13.6, 7.5 Hz, *p*-MeOPhCHH), 3.16 (1H, d, *J* = 11.9 Hz, CHHOH), 3.49 (6H, br. s, NMe₂), 3.68 (1H, irregular t, *J* = 7.5, 6.8 Hz, Hα-tyrosyl), 3.77 (3H, s, OCH₃), 3.86 (1H, d, *J* = 11.9 Hz, CHHOH), 4.77–4.87 (1H, m, H-2'), 5.01 (1H, d, *J* = 6.6 Hz, H-4'), 6.88 (2H, d, *J* = 8.6, Ph(OMe)), 7.16 (2H, d, *J* = 8.6 Hz, Ph(OMe)), 8.19 (1H, s, H-2), 8.55 (1H, s, H-8); ¹³C NMR (CD₃OD, 75 MHz) 10.7, 26.5, 36.0, 37.0, 39.1, 40.6, 50.2, 55.8, 56.4, 57.1, 63.5, 115.2, 121.2, 129.5, 131.5, 139.2, 150.7, 152.8, 156.2, 160.3, 174.9; HRMS (ESI⁺) calcd for (C₂₄H₃₂N₇O₃)⁺ 466.2567 (MH⁺), found 466.2566.

(2S)-N-[(1R,2S,4S,5S)-4-[6-[1-Aza-2-(dibutylamino)-vinyl]purin-9-yl]-1-(hydroxymethyl)bicyclo[3.1.0]hex-2-yl]-2-[(fluoren-9-ylmethoxy)carbonylamino]-3-(4-methoxyphenyl)propanamide (23). A mixture of *N*-Fmoc-*O*-Me-L-Tyr (99 mg, 0.247 mmol) and HOBt (40 mg, 0.247 mmol) was coevaporated three times with anhydrous THF (3 mL). The residue was dissolved in THF (2.5 mL), and the solution was cooled to 0 °C under N₂ for 10 min. Then, DIC (34 μL, 0.212 mmol) was added, and the reaction mixture was stirred for 10 min at the same temperature. Nearby, (Me)₃P (1 M in THF, 282 μL, 0.282 mmol) was added to a solution of the azide **20** (75 mg, 0.176 mmol) in THF (2.5 mL), and the

mixture was stirred for 1 min at room temperature. The amino acid solution was warmed to room temperature during 1 min and then added to the iminophosphorane solution. The reaction mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure, coevaporated from CHCl₃ (10 mL), dissolved in EtOAc (30 mL), and extracted with saturated NaHCO₃ (15 mL). The organic layer was extracted twice with EtOAc, washed with H₂O (2 × 10 mL), dried (MgSO₄), filtered, and evaporated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/cyclohexane/MeOH, 3/1/0 → 4/1/0.5 → 4/1/0.7, v/v) to give the coupling product **23** (115 mg, 82%) as a solid, mp 99–102 °C.

(2S)-N-[(1R,2S,4S,5S)-4-(6-Aminopurin-9-yl)-1-(hydroxymethyl)-bicyclo[3.1.0]hex-2-yl]-2-amino-3-(4-methoxyphenyl)propanamide (5). Compound **23** (57 mg, 0.071 mmol) was dissolved in 33% CH₃NH₂/EtOH (7 mL). The reaction mixture was stirred at room temperature overnight in a closed vessel. The solution was concentrated under reduced pressure and coevaporated from CHCl₃ (7 mL). The oily residue was purified by silica gel column chromatography (EtOAc/toluene/MeOH, 5/1/0.5 → 4/1/0.5; EtOAc/MeOH/H₂O, 8/1/0.5 → 6/1/0.5 → 4/1/0.5, v/v) to give after evaporation the target compound (**5**) as a fluffy solid (30 mg, 96%), which after lyophilization from H₂O/TFA (pH 3.0) gave the more water-soluble salt **5**·TFA: mp 140–142 °C; ¹H NMR (D₂O, 500 MHz) δ 0.69 (1H, irregular t, *J* = 7.0, 8.0, H-6'a), 0.81 (1H, dd, *J* = 6.0, 4.0 Hz, H-6'b), 1.66–1.74 (2H, m, H-5', H-3'a), 2.11 (1H, dd, *J* = 10.0, 8.0 Hz, H-3'b), 2.82 (1H, dd, 1 H, *J* = 13.5, 6.9 Hz, *p*-MeOPhCHH), 3.02–3.08 (1H, m, *p*-MeOPhCHH), 3.07 (1H, d, *J* = 12.3 Hz, CHHOH), 3.49 (1H, d, *J* = 12.3 Hz, CHHOH), 3.75–3.82 (1H, m, Hα-tyrosyl), 3.82 (3H, s, OCH₃), 4.61 (1H, irregular t, *J* = 9.5, 8.5 Hz, H-2'), 4.86 (1H, d, *J* = 8.5 Hz, H-4'), 6.95 (2H, d, *J* = 8.5 Hz, Ph(OMe)), 7.18 (2H, d, *J* = 8.5 Hz, Ph(OMe)), 8.10 (1H, s, H-2), 8.34 (1H, s, H-8); ¹³C NMR (C₅D₅N/few drops D₂O, 75 MHz) δ 10.6, 25.8, 35.8, 36.3, 41.3, 49.7, 55.8, 55.9, 57.4, 63.0, 114.7, 119.7, 130.6, 131.4, 139.9, 149.2, 153.0, 156.5, 158.9, 176.9; HRMS (ESI⁺) calcd for (C₂₂H₂₈N₇O₃)⁺ 438.2254 (MH⁺), found 438.2252.

(2S)-N-[(1S,3S,4S,5S)-1-[6-(Dimethylamino)purin-9-yl]-4-(hydroxymethyl)bicyclo[3.1.0]hex-3-yl]-2-(fluoren-9-ylmethoxy)-carbonylamino]-3-(4-methoxyphenyl)propanamide (24). A mixture of *N*-Fmoc-*O*-Me-L-Tyr (106 mg, 0.263 mmol) and HOBt (42 mg, 0.263 mmol) was coevaporated three times from anhydrous THF (2 mL). The residue was dissolved in THF (2.0 mL), and the solution was cooled to 0 °C under N₂ for 10 min. Then, DIC (36 μL, 0.225 mmol) was added, and the reaction mixture was stirred for 10 min at the same temperature. Nearby, (Me)₃P (1 M in THF, 375 μL, 0.375 mmol) was added to a solution of the azide **6** (59 mg, 0.188 mmol) in THF (2.0 mL) and stirred for 1 min at room temperature. The amino acid was warmed to room temperature during 1 min and then added to the iminophosphorane solution. The reaction mixture was stirred at room temperature overnight. The solution was concentrated under reduced pressure, coevaporated from CHCl₃ (10 mL), then dissolved in EtOAc (30 mL), and extracted with saturated NaHCO₃ (15 mL). The organic layer was extracted twice with EtOAc, washed with H₂O (2 × 10 mL), dried (MgSO₄), filtered, and evaporated in vacuo. The oily residue was purified by silica gel column chromatography (EtOAc/cyclohexane/MeOH, 2/1/0 → 3/1/0 → 4/1/0 → 5/1/0 → 4/1/0.5, v/v) to give the coupling product **24** (105 mg, 81%) as a colorless resin.

(2S)-N-[(1S,3S,4S,5S)-1-[6-(Dimethylamino)purin-9-yl]-4-(hydroxymethyl)bicyclo[3.1.0]hex-3-yl]-3-(4-methoxyphenyl)propanamide (8). The protected coupling product **24** (60 mg, 0.087 mmol) was dissolved in 33% CH₃NH₂/EtOH (5 mL). The reaction mixture was stirred at room temperature overnight in a closed vessel. The solution was concentrated under reduced pressure and coevaporated from CHCl₃ (7 mL), and the oily residue was purified by silica gel column chromatography [(EtOAc/toluene/MeOH), 5/1/0.5 → 4/1/0.5, v/v; (EtOAc/MeOH/H₂O), 8/1/0.5 → 6/1/0.5 → 4/1/0.5, v/v] to give after evaporation the target compound **8** (39 mg,

96%) and, after lyophilization from H₂O/TFA (pH 3.0), the more water-soluble salt **8**•TFA as a off-white amorphous and hygroscopic solid: ¹H NMR (CD₃OD, 500 MHz) δ 0.68 (1H, dd, *J* = 5.4, 6.3 Hz, H-6'a), 1.23–1.32 (1H, m, H-6'b), 1.79–1.90 (2H, m, H-5', H-4'), 2.24 (1H, d, *J* = 13.5 Hz, H-2'a), 2.56 (1H, dd, *J* = 13.5, 9.0 Hz, H-2'b), 2.99 (1H, dd, *J* = 9.6, 12.4 Hz, *p*-MeOPhCHH), 3.18 (1H, dd, *J* = 5.7, 12.4 Hz, *p*-MeOPhCHH), 3.55 (6H, br s, NMe₂), 3.80 (3H, s, OCH₃), 3.83 (1H, dd, *J* = 4.8, 11.4 Hz, CHHOH), 3.91 (1H, dd, *J* = 3.9, 11.4 Hz, CHHOH), 3.96 (1H, dd, *J* = 5.7, 9.6 Hz, Hα-tyrosyl), 4.14 (1H, d, *J* = 9.0 Hz, H-3'), 6.88 (2H, d, *J* = 8.6 Hz, Ph(OMe)), 7.13 (2H, d, *J* = 8.6 Hz, Ph(OMe)), 7.98 (1H, s, H-8), 8.19 (1H, s, H-2); ¹³C NMR (CD₃OD, 125 MHz) δ 18.0, 28.4, 39.1, 39.8, 40.6, 44.7, 51.6, 54.9, 55.8, 56.5, 66.4, 115.3, 121.2, 128.8, 131.7, 141.2, 151.7, 152.8, 156.3, 160.6, 172.3; HRMS (ESI⁺) calcd for (C₂₄H₃₁N₇NaO₃)⁺ 488.2386 (MNa⁺), found 488.2389.

(2S)-N-((1S,3S,4S,5S)-1-{6-[1-Aza-2-(dibutylamino)vinyl]purin-9-yl}-4-(hydroxymethyl)bicyclo[3.1.0]hex-3-yl)-2-(fluoren-9-ylmethoxy)carbonylamino]-3-(4-methoxyphenyl)propanamide (25). *N*-Fmoc-O-Me-L-Tyr (106 mg, 0.263 mmol) and HOBT (42 mg, 0.263 mmol) was coevaporated three times from anhydrous THF (3 mL). The mixture was dissolved in THF (2.0 mL), and the solution was cooled to 0 °C under N₂ for 10 min. Then, DIC (36 μL, 0.226 mmol) was added, and the reaction mixture was stirred for 10 min at the same temperature. Nearby, (Me)₃P (1 M in THF, 376 μL, 0.376 mmol) was added to a solution of the azide **21** (79 mg, 0.186 mmol) in THF (2.0 mL) and stirred for 1 min at room temperature. The aminoacid solution was warmed to room temperature during 1 min and then added to the iminophosphorane solution. The reaction mixture was stirred at room temperature overnight. The solution was concentrated in vacuo, coevaporated from CHCl₃ (10 mL), then dissolved in EtOAc (30 mL), and extracted with saturated NaHCO₃ (15 mL). The organic layer was extracted twice with EtOAc, washed with H₂O (2 × 10 mL), dried (MgSO₄), filtered, and evaporated under reduced pressure. The oily residue was purified by silica gel column chromatography (EtOAc/cyclohexane/MeOH, 2/1/0→3/1/0→4/1/0.5→4/1/0.7, v/v) to give the coupling product **25** (120 mg, 81%) as a white solid, mp 91–93 °C.

(2S)-N-[(1S,3S,4S,5S)-1-(6-Aminopurin-9-yl)-4-(hydroxymethyl)bicyclo[3.1.0]hexan-3-yl]-2-amino-3-(4-methoxy-phenyl)-

propanamide (9). Compound **25** (59 mg, 0.074 mmol) was dissolved in 33% CH₃NH₂/EtOH (5 mL). The reaction mixture was stirred at room temperature overnight in a closed vessel. The solution was then concentrated under reduced pressure and co-evaporated from CHCl₃ (8 mL). The oily residue was purified by silica gel column chromatography (EtOAc/toluene/MeOH, 5/1/0.5→4/1/0.5, v/v; EtOAc/MeOH/H₂O, 8/1/0.5→6/1/0.5→4/1/0.5, v/v) to give after evaporation the target compound **9** (31 mg, 96%) and, after lyophilization from H₂O/TFA (pH 3.0), the more water-soluble salt **9**•TFA as an off-white and hygroscopic amorphous solid: ¹H NMR (CD₃OD, 300 MHz) δ 0.80 (1H, irregular t, *J* = 5.1, 6.3 Hz, H-6'a), 1.14–1.27 (1H, m, H-6'b); 1.69 (1H, dd, *J* = 4.8, 9.6 Hz, H-5'), 1.85–1.89 (1H, m, H-4'), 2.16 (1H, d, *J* = 13.8 Hz, H-2'a), 2.46–2.54 (1H, m, H-2'b); 2.84–3.00 (2H, m, *p*-MeOPhCH₂), 3.68 (3H, s, OCH₃), 3.75 (1H, d, *J* = 11.3, 4.1 Hz, CHHOH), 3.80–3.90 (1H, m, Hα-tyrosyl), 3.86 (1H, dd, *J* = 11.3, 3.0 Hz, CHHOH); 4.13 (1H, d, *J* = 8.7 Hz, H-3'), 6.82 (2H, d, *J* = 8.4 Hz, Ph(OMe)), 7.11 (2H, d, *J* = 8.4 Hz, Ph(OMe)), 8.04 (1H, s, H-8), 8.08 (1H, s, H-2); ¹³C NMR (CD₃OD, 75 MHz) δ 18.1, 28.3, 39.1, 40.5, 44.8, 51.6, 54.9, 55.8, 56.2, 66.3, 115.4, 120.2, 128.3, 131.7, 143.2, 150.9, 153.5, 157.5, 160.6, 171.3; HRMS (ESI⁺) calcd for (C₂₂H₂₇N₇NaO₃)⁺ 460.2073 (MNa⁺), found 460.2072.

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Supporting Information Available: General experimental details, procedures for the synthesis of compounds in Schemes 2 and 3, and record of ¹H NMR, ¹³C NMR, and HRMS for compounds **22**–**25**, plus full NMR spectral characterization of all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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