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Synthesis of diastereomeric 3-hydroxy-4-pyrrolidinyl derivatives of nucleobases

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Abstract—The work deals with the synthesis of hydroxypyrrolidine analogs of nucleosides. Starting from the optically pure L- or D-tartaric acid, we improved the synthesis of enantiomeric *trans*-3,4-dihydroxypyrrolidines and elaborated a procedure for the synthesis of all possible diastereoisomers of 3-hydroxy-4-pyrrolidinyl derivatives of both purine and pyrimidine nucleobases. The prepared compounds were tested for cytostatic and antiviral properties but no significant activity was found.

1. Introduction

Sugar-modified nucleoside analogs form a large group of potential antimetabolites.[1] Thus, 1,3-oxothiolane,[2] 1,3-dioxolane,[3–6] and cyclobutane[7] and cyclopentane[8–12] ring-containing D- and L-nucleoside analogs have been synthesized and biologically evaluated. Among these analogs, potent compounds exhibiting remarkable antiviral and anticancer properties have been found.[13]

Replacement of the sugar moiety in nucleosides by a pyrrolidine ring seems to be one of the promising modifications, which could provide compounds possessing both diverse biological activities and the possibility of further derivatization, e.g., on the nitrogen atom of the pyrrolidine ring (Fig. 1).

The pyrrolidine nucleosides have attracted the attention of several laboratories.[14–26] Thus, Miyabe et al.[23] reported the total synthesis of 3-hydroxy-4-pyrrolidinyl derivatives of uracil, thymine, and adenine **1** via construction of both the pyrrolidine ring and the nucleobase moiety; in this case racemic mixtures were prepared. Richichi et al.[24] described the preparation of protected 3-hydroxypyrrolidinyl derivatives of uracil **2c** and thymine **2d** by Mitsunobu reaction of unprotected pyrimidine bases with appropriate *N*-benzylpyrrolidin-3,4-diols. Recently, we published the synthesis of 3-pyrrolidinyl derivatives of all four nucleobases mimicking 2′,3′-dideoxynucleosides, by nucleophilic displacement of the mesyloxy group for individual nucleobases under various conditions.[25,26]

In this paper, we describe the synthesis of diastereomeric 3-hydroxy-4-pyrrolidinyl derivatives of thymine and adenine by nucleophilic displacement of mesyloxy group for nucleobase.

2. Results and discussion

The enantiomeric *trans*-3,4-dihydroxypyrrolidines **8a** and **8b** are known compounds and their synthesis starting from

1a *3R, 4R; trans*; B= T
1b *3R, 4R; trans*; B= A
1c *3R, 4R; trans*; B= U

2a *3S, 4R; cis*; B= T; R= H
2b *3S, 4R; cis*; B= A; R= H
2c *3R, 4S; cis*; B= U; R= Bn
2d *3R, 4S; cis*; B= T; R= Bn

3a *3S, 4S; trans*; B= T
3b *3S, 4S; trans*; B= A

4a *3R, 4S; cis*; B= T
4b *3R, 4S; cis*; B= A

Figure 1.

Keywords: Tartaric acid; Pyrrolidine derivatives; Nucleoside analogs; Nucleobase.
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L- and D-tartaric acids (**5a** and **5b**, respectively) was described in the literature many times.[27–35] We prepared **8a** and **8b** from the appropriate tartaric acids via corresponding 2,5-diones **6a** and **6b**, the preparation of which was recently improved.[26] In short, the monobenzylammonium salt of tartaric acid prepared in aqueous methanol was refluxed, after evaporation of solvents, in xylene in the Dean–Stark apparatus for 8 h. A clear, homogenous solution was obtained within several minutes of heating. Xylene was evaporated in vacuo and the crude material was crystallized from hot ethanol to yield the products **6a** and **6b** in ~90% yield (Scheme 1). The reduction of benzyl imides **6a** and **6b** to benzylpyrrolidines **7a** and **7b**, respectively, proceeded smoothly by treatment with diborane generated in situ from $NaBH_4$ and iodine in THF.[36] Desalting of *N*-benzyl-3-pyrrolidinols **7a** and **7b** on Dowex 50W (H^+) followed by removal of *N*-benzyl group using catalytic hydrogenation afforded pure 3-pyrrolidinols **8a** and **8b**, respectively.

Having prepared *N*-benzyl derivative **7**, we first attempted to use it for the protection of one hydroxyl of the *trans* diol moiety (Scheme 2). Thus, **7a** was dimethoxytritylated under various conditions with the aim of increasing the yield of the monosubstituted product **9** (Table 1). As both the hydroxyl groups in **7a** are equally reactive, it is difficult in this case to obtain nearly quantitative yield as it is in the case of non-equivalent hydroxyls (e.g., dimethoxytritylation of primary hydroxyl in the presence of secondary one in nucleosides).

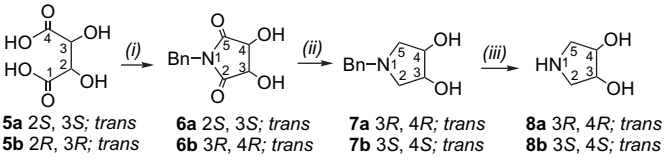
As is obvious from Table 1, the best yield of the dimethoxytrityl derivative **9** was obtained in the first two cases, using pyridine and DCM as solvents. The use of dimethoxytrityl group offers several advantages: (i) it is stable under nucleophilic displacement (alkaline conditions), (ii) the reagent for its introduction (DMTrCl) possesses sufficient reactivity, (iii) the group is bulky enough to react preferentially with only one hydroxyl, and (iv) its removal is accomplished under mild acidic conditions. The subsequent mesylation

Table 1. Conditions for dimethoxytritylation of **7a** to **9** (Scheme 2)

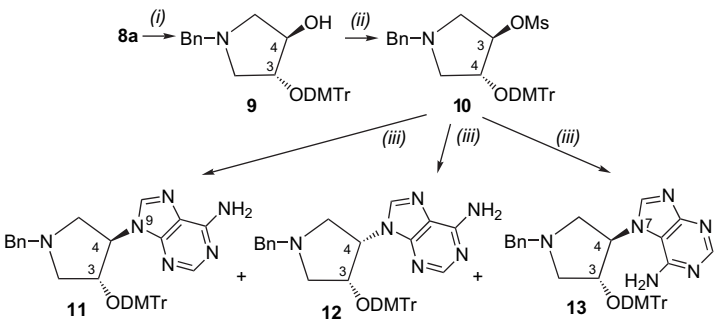
Conditions (rt, 16 h)	Yield, %
DMTrCl (1.5 equiv)/pyridine	56
DMTrCl (1.5 equiv)/DCM/DMAP (1.5 equiv)	54
DMTrCl (1.5 equiv)/THF/NaH (1 equiv)/−78 °C	46
DMTrCl (1.5 equiv)/DBU (1.5 equiv)/(no solvent)	44
DMTrCl (1.5 equiv)/pyridine/DBU (1.5 equiv)	36
DMTrCl (1.5 equiv)/DCM/DBU (1.5 equiv)	25
DMTrCl (2 equiv)/THF/$AgNO_3$ (1.5 equiv)	25

of **9** was fast and nearly quantitative using an excess of mesyl chloride. The mesyl derivative **10** was subjected to the reaction with the cesium salt of adenine in DMSO at elevated temperature. However, the reaction did not proceed via S_N2-substitution mechanism exclusively, so that a diastereomeric mixture of products **11** and **12**, together with the 7-*N*-substituted adenine isomer **13**, were obtained in a low yield. Attempts at tuning the reaction conditions (e.g., the use of DMF and variable amount of sodium hydride) to improve either the yield or at least the product distribution failed. The structure of compounds **11**–**13** was unambiguously confirmed using a combination of NMR techniques (H,H-COSY, H,C-HSQC and H,C-HMBC). The relative configuration of **11** and **12** at chiral carbons C-3 and C-4 was checked by ROESY, whereby NOE contacts between H-8 from adenine and particular protons from the pyrrolidine ring served as the proof of the configuration.

We concluded that the presence of the tertiary amine moiety possessing a free electron pair on pyrrolidine nitrogen atom is likely to be the main factor influencing the epimerization of the C-3 atom of **10** during nucleophilic displacement of the mesyloxy group. The pyrrolidine nitrogen atom could interact with a C-3 carbocation (or with some kind of S_N2 transition state intermediate) via a free electron pair giving rise to two hypothetical chiral intermediates A and B (Fig. 2), which can be attacked on the C-3 atom by adenine as a nucleophile either from the cis or trans position with respect to the dimethoxytrityloxy substituent, providing a mixture of



Scheme 1. (i) (1) $BnNH_2$, methanol; (2) xylene, reflux; (ii) $NaBH_4$, I_2, THF; (iii) H_2, Pd/C, ethanol.



Scheme 2. (i) DMTrCl, pyridine; (ii) MsCl, DMAP, DCM; (iii) adenine, Cs_2CO_3, DMSO.

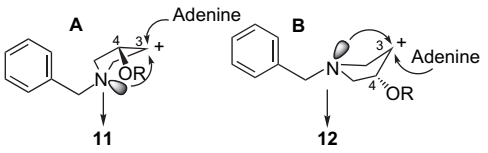


Figure 2. Possible explanation of epimerization (see also Scheme 2).

diastereoisomers **11** and **12**. The formation of a quaternary aziridine derivative as an intermediate does not seem to be plausible.

In order to overcome the problem with participation of the free electron pair of basic pyrrolidine nitrogen atom during the nucleophilic displacement of mesyloxy group in **10** for nucleobase, we exchanged the *N*-benzyl protecting group of pyrrolidine nitrogen atom for a *N*-butyloxycarbonyl group, which made the nitrogen atom non-basic due to delocalization of the electron pair. The *N*-benzyl group of compounds **7a** and **7b** was removed by a catalytic reduction under hydrogen and the unprotected 3,4-dihydroxypyrrolidines **8a** and **8b** were transformed into *N*-Boc derivatives **14a** and **14b**, respectively (Scheme 3). It should be noted that the starting *N*-benzylpyrrolidine **7** had to be passed through silica gel before the hydrogenation step, to avoid poisoning of the palladium catalyst by unidentified impurities.

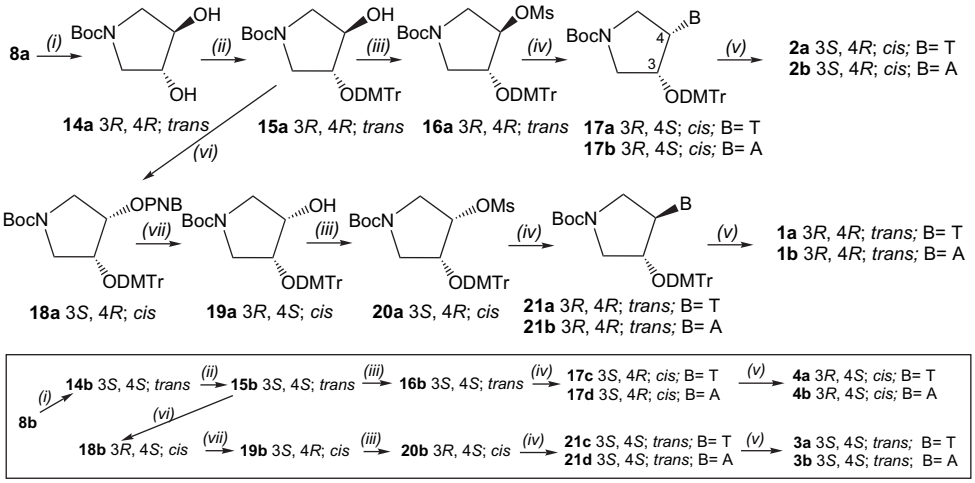
Dimethoxytritylation of the 3-hydroxy group of **14** with dimethoxytrityl chloride in pyridine provided 70% yield of compound **15**, which was mesylated to give the *trans* mesyl derivative **16**. To obtain *cis* mesylate **20**, the configuration on carbon C-3 was inverted using Mitsunobu reaction with 4-nitrobenzoic acid. The ester **18** was treated with methanolic ammonia to remove the 4-nitrobenzoyl group, and subsequent mesylation of **19** provided the *cis* mesyl derivative **20**. The obtained mesylates **16** and **20** were used for the nucleosidation reaction with the cesium or potassium salt of adenine or thymine in DMSO. The nucleosidation reactions led in both cases to a good yield of the desired adenine derivatives and to a moderate yield of the desired thymine ones. Final compounds **1a**,**b** and **3a**,**b** with trans configuration, as well as compounds **4a**,**b** and **2a**,**b** with cis configuration, were obtained by deprotection using 20% TFA in DCM. Again, the relative configurations of the free nucleosides were assigned by ROESY spectra.

All the steps involved in the series of reactions were simple and gave very good yields. The only difficulty encountered was with the purification of 4-nitrobenzoate **18** after Mitsunobu reaction. Residual diisopropyl-hydrazinodicarboxylate was completely removed at the level of mesyl derivative **20** using silica gel chromatography carefully (elution with a linear gradient of toluene in petroleum ether followed by a slow linear gradient of ethyl acetate in toluene).

The cytostatic activity of analogs **1a**,**b**, **2a**,**b**, **3a**,**b**, and **4a**,**b** was examined with L1210, L929, and HeLa S3 cell lines. The antiviral activity was evaluated against (i) HSV-1, HSV-2, vaccinia virus, VSV, and HSV-1 TK$^-$ KOS ACV^r in HEL cell cultures, (ii) VSV, coxsackie virus B4 in HeLa cell cultures, and (iii) parainfluenza virus, rheovirus-1, sindbis virus, coxsackie virus B4, and Punta Toro virus in Vero cell cultures. No significant activity was found.

3. Conclusions

We reported the synthesis of novel nucleoside mimics, eight diastereoisomeric 3-hydroxy-4-pyrrolidinyl derivatives of adenine and thymine **1a**,**b**, **2a**,**b**, **3a**,**b**, and **4a**,**b** by a direct nucleophilic displacement of mesyloxy group in **16a**,**b** and **19a**,**b** for nucleobases. We improved the synthesis of key enantiomeric *trans*-1-*N*-benzyl-3,4-dihydroxypyrrolidines **8a** and **8b** to obtain reproducibly high yields of these compounds. We found that the *N*-benzyl protecting group in mesyloxy derivative **10** participated in the nucleophilic displacement of mesyloxy group for the adenine moiety and that, therefore, this reaction proceeded with epimerization. The prepared compounds **1**–**4** offer further possibility for derivatization at the pyrrolidine nitrogen atom, thus providing the chance to prepare other types of structurally diverse compounds, e.g., phosphonate pyrrolidine nucleotides, and consequently, enlarge the area of potentially biologically active compounds. This makes these compounds interesting,



Scheme 3. (i) Boc_2O, $NaHCO_3$, dioxane, water; (ii) DMTrCl, pyridine; (iii) MsCl, DMAP, DCM; (iv) adenine or thymine, Cs_2CO_3 or K_2CO_3, DMSO; (v) 20% TFA/DCM; (vi) Ph_3P, PNBA, DIAD, THF; (vii) NH_3/methanol.

although no significant cytostatic and antiviral activities of the prepared compounds were observed.

4. Experimental

4.1. General

Unless stated otherwise, all used solvents were anhydrous. Final products were lyophilized from water, and dried over phosphorus pentoxide at 50–70 °C and 13 Pa. TLC was performed on silica gel pre-coated aluminum plates Silica gel/TLC-cards, UV 254 (Fluka), and the compounds were detected by UV light (254 nm), by heating (detection of dimethoxytrityl group; orange color), by spraying with 1% solution of ninhydrin to visualize amines, and by spraying with 1% solution of 4-(4-nitrobenzyl)pyridine in ethanol followed by heating and treating with gaseous ammonia (blue color of mono- and diesters of phosphonic acid). Preparative column chromatography was carried out on silica gel (40–60 μm, Fluka) neutralized with triethylamine (1 mL/100 g), and elution was performed at the flow rate of 40 mL/min. The following solvent systems were used for TLC and preparative chromatography: toluene/ethyl acetate 1:1 (T); chloroform/ethanol 9:1 (C1); ethyl acetate/acetone/ethanol/water 6:1:1:0.5 (H3); ethyl acetate/acetone/ethanol/water 4:1:1:1 (H1). The concentrations of solvent systems are stated in volume percents (%, v/v). Analytical RP-HPLC was performed on LC5000 Liquid Chromatograph (INGOS-PIKRON, CR) using Luna C18 (2) column (4.6×150 mm) at the flow rate of 1 mL/min by a gradient elution of methanol in 0.1 M TEAA, pH 7.5 (A=0.1 M TEAA, B=0.1 M TEAA in 50% aqueous methanol, C=methanol). Mass spectra were recorded on ZAB-EQ (VG Analytical) instrument, using FAB (ionization with Xe, accelerating voltage 8 kV). Glycerol and thioglycerol were used as matrices. NMR spectra were measured on Bruker Avance 400 (^{1}H at 400 MHz, ^{13}C at 100.6 MHz), Bruker Avance 500, and Varian Unity 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) spectrometers. Chemical shifts (in parts per million, δ scale) were referenced to TMS as internal standard; coupling constants (J) are given in Hertz. Complete assignment of protons and carbons was achieved by analysis of correlated homonuclear 2D-COSY and heteronuclear ^{1}H–^{13}C HSQC and ^{1}H–^{13}C HMBC spectra. Relative configuration was checked using DPFGSE-NOE and 2D-ROESY techniques.

4.1.1. (3*R*,4*R*)-3-Hydroxy-4-(thymin-1-yl)-pyrrolidine (1a). Compound **21a** (3.2 g, 5.2 mmol) was treated with a 20% solution of TFA in DCM (50 mL) containing 1% of water overnight. The reaction mixture was concentrated in vacuo, water was added, and the solution was applied onto a column of Dowex 50W in H^+ cycle (100 mL). The resin was washed with water (300 mL) and the desired compound **1a** was obtained after elution with 3% aqueous ammonia, concentration, and lyophilization from water in 68% yield (0.8 g) in the form of a white amorphous solid.

HRMS for $C_9H_{13}N_3O_3$ $(M+H)^+$ calcd: 212.1035, found: 212.1043. ν_{max} (KBr) 3387 (m, br, sh), 3342 (s), 3160 (w, br), 1684 (vs, br), 1664 (vs, sh), 1642 (s, sh), 1492 (m), 1466 (m), 1441 (m), 1400 (m), 1388 (m), 1375 (m), 1284 (m), 1272 (m, sh), 765 (m). ^{1}H NMR (500 MHz, DMSO-d_6): 1.77 (d, 3H, $J_{CH_3,6}=1.2$, CH_3-5), 2.63 (dd, 1H, J_{gem}=11.3, $J_{2'b,3'}$=5.1, H-2′b), 2.75 (dd, 1H, J_{gem}=11.7, $J_{5'b,4'}$=5.7, H-5′b), 3.08 (dd, 1H, J_{gem}=11.3, $J_{2'a,3'}$=6.2, H-2′a), 3.19 (dd, 1H, J_{gem}=11.7, $J_{5'a,4'}$=8.1, H-5′a), 4.16 (dt, 1H, $J_{3',2'}$=6.2, 5.1, $J_{3',4'}$=4.5, H-3′), 4.52 (ddd, 1H, $J_{4',5'}$=8.1, 5.7, $J_{4',3'}$=4.5, H-4′), 7.52 (q, 1H, $J_{6,CH_3}=1.2$, H-6). ^{13}C NMR (125.8 MHz, DMSO-d_6): 12.31 (CH_3-5), 49.73 (CH_2-5′), 53.63 (CH_2-2′), 64.11 (CH-4′), 75.58 (CH-3′), 109.24 (C-5), 138.81 (CH-6), 151.27 (C-2), 164.05 (C-4).

4.1.2. (3*R*,4*R*)-4-(Adenin-9-yl)-3-hydroxypyrrolidine (1b). Compound **1b** was prepared from **21b** (7.7 g, 12.4 mmol) using the same procedure as for the compound **1a** in 84% yield (2.3 g) in the form of a white amorphous solid.

HRMS for $C_9H_{12}N_6O$ $(M+H)^+$ calcd: 221.1151, found: 221.1151. $[\alpha]_D^{20}$ −18.7 (*c* 0.171, H_2O); ν_{max} (KBr) 3455 (m), 3410 (m, br, sh), 3360 (m, sh), 3336 (s), 3325 (s, sh), 3267 (m), 3210 (s), 1658 (vs), 1637 (s, sh), 1608 (s), 1573 (m), 1506 (w), 1479 (m), 1414 (m), 1374 (w), 1330 (m), 1311 (m), 1296 (w), 1256 (m), 1225 (w), 797 (w), 653 (m), 538 (w). ^{1}H NMR (500 MHz, DMSO-d_6): 2.72 (dd, 1H, J_{gem}=11.6, $J_{2'b,3'}$=4.5, H-2′b), 3.05 (dd, 1H, J_{gem}=11.7, $J_{5'b,4'}$=5.3, H-5′b), 3.25 (dd, 1H, J_{gem}=11.6, $J_{2'a,3'}$=6.2, H-2′a), 3.35 (dd, 1H, J_{gem}=11.7, $J_{5'a,4'}$=7.3, H-5′a), 4.42 (dt, 1H, $J_{3',2'}$=6.2, 4.5, $J_{3',4'}$=3.8, H-3′), 4.65 (ddd, 1H, $J_{4',5'}$=7.3, 5.3, $J_{4',3'}$=3.8, H-4′), 7.17 (br s, 2H, NH_2), 8.13 (s, 1H, H-2), 8.17 (s, 1H, H-8). ^{13}C NMR (125.8 MHz, DMSO-d_6): 50.98 (CH_2-5′), 53.89 (CH_2-2′), 63.23 (CH-4′), 76.49 (CH-3′), 119.14 (C-5), 139.70 (CH-8), 149.51 (C-4), 152.36 (CH-2), 156.16 (C-6).

4.1.3. (3*S*,4*R*)-3-Hydroxy-4-(thymin-1-yl)-pyrrolidine (2a). Compound **2a** was prepared from **17c** (3.7 g, 6 mmol) using the same procedure as for the compound **1a** in 73% yield (1 g) in the form of a white amorphous solid.

HRMS for $C_9H_{12}N_6O$ $(M+H)^+$ calcd: 212.1035, found: 221.1013. $[\alpha]_D^{20}$ +73.6 (*c* 0.324, H_2O); ν_{max} (KBr) 3393 (m), 3317 (m), 1696 (vs, br), 1665 (vs), 1643 (m, sh), 1489 (m), 1468 (m), 1444 (m), 1394 (m), 1375 (w), 1295 (m), 1271 (m), 1076 (w), 1060 (m), 767 (m), 763 (m). ^{1}H NMR (400 MHz, DMSO-d_6): 1.76 (d, 3H, $J_{CH_3,6}=1.2$, CH_3-5), 2.69 (dd, 1H, J_{gem}=12.0, $J_{2'b,3'}$=3.0, H-2′b), 2.94 (dd, 1H, J_{gem}=11.5, $J_{5'b,4'}$=8.0, H-5′b), 3.01 (dd, 1H, J_{gem}=11.5, $J_{2'a,3'}$=8.0, H-2′a), 3.08 (dd, 1H, J_{gem}=12.0, $J_{5'a,4'}$=5.6, H-5′a), 4.11 (td, 1H, $J_{3',4'}$=6.1, $J_{3',2'}$=5.6, 3.0, H-3′), 4.68 (td, 1H, $J_{4',5'}$=8.0, $J_{4',3'}$=6.1, H-4′), 5.07 (br, 1H, OH), 7.48 (q, 1H, $J_{CH_3,6}=1.2$, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): 12.37 (CH_3-5), 48.13 (CH_2-5′), 54.55 (CH_2-2′), 57.14 (CH-4′), 69.19 (CH-3′), 107.01 (C-5), 140.53 (CH-6), 151.71 (C-2), 164.10 (C-4).

4.1.4. (3*S*,4*R*)-4-(Adenin-9-yl)-3-hydroxypyrrolidine (2b). Compound **2b** was prepared from **17d** (6 g, 9.6 mmol) using the same procedure as for the compound **1a** in 75% yield (1.6 g) in the form of a white amorphous solid.

HRMS for $C_9H_{12}N_6O$ $(M+H)^+$ calcd: 221.1151, found: 221.1013. $[\alpha]_D^{20}$ +91.2 (*c* 0.339, H_2O); ν_{max} (KBr) 3433

(m, br, sh), 3319 (s), 3266 (s), 3177 (s, br), 1644 (vs, br), 1600 (vs), 1575 (s), 1505 (w), 1475 (s), 1415 (s), 1370 (m), 1331 (m), 1301 (m), 1254 (m), 1220 (m), 1081 (m, sh), 1067 (m, br), 798 (m), 649 (m), 536 (w). ^{1}H NMR (400 MHz, DMSO-d_6): 2.79 (dd, 1H, J_{gem}=11.9, $J_{2'b,3'}$=3.3, H-2′b), 3.17 (dd, 1H, J_{gem}=11.1, $J_{5'b,4'}$=8.4, H-5′b), 3.21 (dd, 1H, J_{gem}=11.9, $J_{2'a,3'}$=5.6, H-2′a), 3.23 (dd, 1H, J_{gem}=11.1, $J_{5'a,4'}$=8.2, H-5′a), 4.21 (dt, 1H, $J_{3',4'}$=5.8, $J_{3',2'}$=5.6, 3.3, H-3′), 4.81 (dt, 1H, $J_{4',5'}$=8.4, 8.2, $J_{4',3'}$=5.8, H-4′), 5.09 (br, 1H, OH), 7.16 (br s, 2H, NH_2), 8.13 (s, 1H, H-2), 8.14 (s, 1H, H-8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 49.58 (CH_2-5′), 54.52 (CH_2-2′), 57.04 (CH-4′), 69.83 (CH-3′), 118.49 (C-5), 140.85 (CH-8), 150.19 (C-4), 152.28 (CH-2), 156.05 (C-6).

4.1.5. (3*S*,4*S*)-3-Hydroxy-4-(thymin-1-yl)-pyrrolidine (3a). Compound **3a** was prepared from **21c** (2.9 g, 4.7 mmol) using the same procedure as for the compound **1a** in 75% yield (0.7 g) in the form of a white amorphous solid.

HRMS for $C_9H_{13}N_3O_3$ (M+H)$^+$ calcd: 212.1035, found: 212.1041. $[\alpha]_D^{20}$ +53.2 (*c* 0.348, H_2O). ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **1a**.

4.1.6. (3*S*,4*S*)-4-(Adenin-9-yl)-3-hydroxypyrrolidine (3b). Compound **3b** was prepared from **21d** (3.5 g, 6.1 mmol) using the same procedure as for the compound **1a** in 78% yield (1 g) in the form of a white amorphous solid.

HRMS for $C_9H_{12}N_6O$ (M+H)$^+$ calcd: 221.1151, found: 221.1154. $[\alpha]_D^{20}$ +53.3 (*c* 0.176, H_2O). ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **1b**.

4.1.7. (3*R*,4*S*)-3-Hydroxy-4-(thymin-1-yl)-pyrrolidine (4a). Compound **3a** was prepared from **17a** (0.34 g, 0.56 mmol) using the same procedure as for the compound **1a** in 88% yield (104 mg, 0.5 mmol) in the form of a white amorphous solid.

HRMS for $C_9H_{13}N_3O_3$ (M+H)$^+$ calcd: 212.1035, found: 212.1032. $[\alpha]_D^{20}$ −101.0 (*c* 0.302, H_2O); ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **2a**.

4.1.8. (3*R*,4*S*)-4-(Adenin-9-yl)-3-hydroxypyrrolidine (4b). Compound **4b** was prepared from **17b** (3.1 g, 5 mmol) using the same procedure as for the compound **1a** in 54% yield (0.6 g) in the form of a white amorphous solid.

HRMS for $C_9H_{12}N_6O$ (M+H)$^+$ calcd: 221.1151, found: 221.1143. $[\alpha]_D^{20}$ −53.5 (*c* 0.213, H_2O). ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **2b**.

4.1.9. (3*S*,4*S*)-1-*N*-Benzyl-3,4-dihydroxypyrrolidin-2,6-dione (6a). Benzylamine (160.7 mL, 1.5 mol) was slowly added to a stirred suspension of (3*S*,4*S*)-tartaric acid (225.1 g, 1.5 mol) in 50% aqueous methanol (300 mL). The resulting mixture was heated at 50 °C until a clear solution was obtained. The viscous solution was concentrated on rotary evaporator, xylene (4 L) was added, and the reaction mixture was refluxed in a Dean–Stark apparatus in oil bath at 190 °C for 8 h. During that period, additional xylene (2×500 mL) was added. The resulting solution was cooled and concentrated in vacuo. The obtained solid material was co-evaporated with ethanol (2×200 mL) to remove traces of xylene and refluxed in ethanol (1000 mL) for 5 min. The suspension was cooled, crystals were filtered off, washed with ethanol (3×100 mL) and dried to yield 242.2 g (73%) of compound **6a**. Combined filtrates were concentrated to a volume of 500 mL. Charcoal (50 g) was added and the suspension was refluxed for 5 min and then filtered through Celite®. The filtration cake was washed with hot ethanol (100 mL) and combined filtrates were left aside to crystallize. The crystallization of mother liquor was repeated three times to obtain an additional 66.4 g (20%) of compound **6a**. The total yield of **6a** was 308.6 g (93%).

Mp 194–197 °C. HRMS for $C_{11}H_{12}N_1O_4$ (M+H)$^+$ calcd: 222.0766, found: 222.0769. ^{1}H NMR (500 MHz, DMSO-d_6): 4.38 (d, 2H, *J*=4.9, OCH), 4.53 and 4.58 (2×d, 2×1H, J_{gem}=15.0, NCH_2), 6.29 (d, 2H, *J*=4.9, OH), 7.24 (m, 2H, H-*m*-Ph), 7.27 (m, 1H, H-*p*-Ph), 7.33 (m, 2H, H-*o*-Ph). ^{13}C NMR (125.8 MHz, DMSO-d_6): 41.31 (NCH_2), 74.65 (OCH), 127.64, 127.67 and 128.69 (CH-Ph), 136.13 (C-*i*-Ph), 174.71 (CO).

4.1.10. (3*R*,4*R*)-1-*N*-Benzyl-3,4-dihydroxypyrrolidin-2,6-dione (6b). Compound **6b** was prepared from (3*R*,4*R*)-tartaric acid (225.1 g, 1.5 mol) using the same procedure as for the compound **6a**. The total yield of **6b** was 298.5 g (90%).

Mp 196–198 °C. HRMS for $C_{11}H_{12}N_1O_4$ (M+H)$^+$ calcd: 222.0766, found: 222.0765. ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **6a**.

4.1.11. (3*R*,4*R*)-1-*N*-Benzyl-3,4-dihydroxypyrrolidine (7a). A solution of iodine (95.2 g, 375 mmol) in THF (450 mL) was added dropwise to a vigorously stirred ice bath cooled suspension of $NaBH_4$ (28.4 g, 750 mmol) in a solution of **6a** (33.2 g, 150 mmol) in THF (700 mL) under argon atmosphere. The reaction mixture was stirred at rt overnight, then cooled to 0 °C, and the excess of $NaBH_4$ was decomposed with 3 M HCl (143 mL) (*note: intensive gas evolution occurred*). The reaction mixture was concentrated in vacuo, filtered through Celite®, the cake was washed with acetone, and the filtrate was evaporated. The oily residue was deionized on Dowex 50W (H^+, 1.5 L). Crude material was purified on a silica gel column using a linear gradient of system H1 in ethyl acetate yielding 27.2 g (94%) of yellowish crystals of compound **7a**.

Mp 86–89 °C. HRMS for $C_{11}H_{16}N_1O_2$ (M+H)$^+$ calcd: 194.1181, found: 194.1186. ^{1}H NMR (500 MHz, DMSO-d_6): 2.30 (dd, 2H, J_{gem}=9.5, J_{vic}=4.5, CH_bH$_a$N), 2.74 (dd, 2H, J_{gem}=9.5, J_{vic}=5.9, CH_aH$_b$N), 3.46 and 3.57 (2×d, 2×1H, J_{gem}=13.1, CH_2-Ph), 3.84 (br t, 2H, *J*=5.9, 4.5, OCH), 4.84 (br s, 2H, OH), 7.20–7.35 (m, 5H, Ph). ^{13}C NMR (125.8 MHz, DMSO-d_6): 60.14 (CH_2-Ph), 60.99 (NCH_2), 77.81 (OCH), 126.92, 128.32 and 128.71 (CH-Ph), 139.23 (C-*i*-Ph).

4.1.12. (3*S*,4*S*)-1-*N*-Benzyl-3,4-dihydroxypyrrolidine (7b). Compound **7b** was prepared from **6b** (33.2 g, 150 mmol) using the same procedure as for the compound **7a** in 93% (26.9 g) yield.

Mp 95–98 °C. HRMS for $C_{11}H_{16}N_1O_2$ $(M+H)^+$ calcd: 194.1181, found: 194.1177. ^1H NMR and ^{13}C NMR spectra were identical to those of compound **7a**.

4.1.13. (3*R*,4*R*)-3,4-Dihydroxypyrrolidine (8a). *N*-Benzylpyrrolidine derivative **7a** (29.0 g, 150 mmol) in 80% aqueous ethanol (900 mL) was treated with hydrogen gas (10 psi) in the presence of 10% Pd/C (2 g) at rt for 2 d. The catalyst was filtered off, the filtrate was concentrated in vacuo, the residue was co-evaporated with ethanol (2×100 mL), and dried over P_2O_5 (13 Pa). Compound **8a** was obtained as a yellow oil in 90% yield (13.9 g).

HRMS for $C_4H_{10}N_1O_2$ $(M+H)^+$ calcd: 104.0712, found: 104.0674. ^1H NMR (500 MHz, DMSO-d_6): 2.60 (m, 2H, CH_bH$_a$N), 3.02 (m, 2H, CH_aH$_b$N), 3.83 (br m, 2H, OCH), 4.81 (br s, 3H, NH+OH). ^{13}C NMR (125.8 MHz, DMSO-d_6): 52.55 (NCH_2), 77.39 (OCH).

4.1.14. (3*S*,4*S*)-3,4-Dihydroxypyrrolidine (8b). Compound **8b** was prepared from **7b** (29.0 g, 150 mmol) using the same procedure as for the compound **8a** in 92% overall yield (14.2 g) in the form of a yellow oil.

HRMS for $C_4H_{10}N_1O_2$ $(M+H)^+$ calcd: 104.0712, found: 104.0674. ^1H NMR and ^{13}C NMR spectra were identical to those of compound **8a**.

4.1.15. (3*R*,4*R*)-1-*N*-Benzyl-3-dimethoxytrityloxy-4-hydroxypyrrolidine (9). Dimethoxytrityl chloride (21.5 g, 64.4 mmol) was added to a solution of **8a** (8.4 g, 43 mmol) in pyridine (500 mL) and the reaction mixture was stirred at rt for 2 d. Anhydrous methanol (10 mL) was added and the solution was concentrated in vacuo. Compound **9** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 56% yield (11.9 g) in the form of a yellow foam.

HRMS for $C_{32}H_{33}NO_4$ $(M+H)^+$ calcd: 495.2420, found: 495.2414. ^1H NMR (500 MHz, DMSO-d_6): 1.55 (dd, 1H, J_{gem}=10.5, $J_{2b,3}$=4.5, H-2b), 1.70 (dd, 1H, J_{gem}=10.5, $J_{2a,3}$=6.7, H-2a), 2.22 (dd, 1H, J_{gem}=9.5, $J_{5b,4}$=4.8, H-5′b), 2.63 (dd, 1H, J_{gem}=9.5, $J_{5a,4}$=6.3, H-5a), 3.31 and 3.34 (2×d, 1H, J_{gem}=13.1, CH_2-Ph), 3.71 (s, 6H, CH_3O-DMTr), 3.78 (m, 1H, H-3), 4.13 (m, 1H, H-3), 4.89 (d, 1H, $J_{OH,4}$=6.0, OH-4), 6.85 (m, 4H, H-*m*-C_6H_4-DMTr), 7.10 (m, 2H, H-*o*-Bn), 7.15–7.30 (m, 10H, H-*o*-C_6H_4-DMTr, H-*o*,*m*,*p*-C_6H_5-DMTr, H-*p*-Bn), 7.45 (m, 2H, H-*m*-Bn). ^{13}C NMR (100.6 MHz, DMSO-d_6): 55.185 (CH_3O-DMTr), 58.99 (CH_2-2), 59.81 (CH_2-5), 60.28 (CH_2-Ph), 76.77 (CH-3), 80.59 (CH-4), 86.00 (C-DMTr), 113.25 and 113.29 (CH-*m*-C_6H_4-DMTr), 126.71, 126.81, 127.88, 128.08 and 128.62 (CH-Bn, CH-C_6H_5-DMTr), 130.02 and 130.14 (CH-*o*-C_6H_4-DMTr), 136.48 and 136.93 (C-*i*-C_6H_4-DMTr), 138.66 (C-*i*-C_6H_5-DMTr), 145.92 (C-*i*-Bn), 158.25 (C-*p*-C_6H_4-DMTr).

4.1.16. (3*R*,4*R*)-1-*N*-Benzyl-4-dimethoxytrityloxy-3-mesyloxypyrrolidine (10). Mesyl chloride (3.6 mL, 46.5 mmol) was added dropwise to the solution of **9** (11.5 g, 23 mmol) and DMAP (5.7 g, 46.5 mmol) in dichloromethane (120 mL) at 0 °C. The reaction mixture was stirred at rt for 2 h, cooled in an ice bath, and quenched with water (10 mL). The reaction mixture was washed with a saturated solution of sodium hydrogen carbonate. The organic layer was dried over sodium sulfate and evaporated. Compound **10** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 83% yield (11 g) in the form of a yellowish foam.

HRMS for $C_{33}H_{35}NO_6S$ $(M+H)^+$ calcd: 574.2263, found: 574.2261. ^1H NMR (500 MHz, DMSO-d_6): 2.63 (dd, 1H, J_{gem}=10.2, $J_{5b,4}$=3.7, H-5b), 2.94 (d, 2H, $J_{2,3}$=7.0, 6.6, H-2), 2.95 (dd, 1H, J_{gem}=10.2, $J_{5a,4}$=6.6, H-5a), 3.69 (s, 2H, CH_2-Ph), 4.40 (m, 1H, $J_{4,3}$=6.6, $J_{4,5}$=6.6, 3.7, $J_{4,OH}$=5.0, H-4), 5.03 (q, 1H, $J_{3',2'a}$=$J_{3',2'b}$=$J_{3',4'}$=6.6, H-3′), 5.11 (d, 1H, $J_{OH,4'}$=5.0, OH-4′), 7.15 (br s, 2H, NH_2), 7.24–7.37 (m, 5×Ar-H (N-Bn)), 8.12 (s, 1 H, H-2), 8.16 (s, 1 H, H-8). ^{13}C NMR (125.7 MHz, DMSO-d_6): 54.89 (CH_2-3′), 57.10 (CH-2′), 59.37 (N-CH_2 (N-Bn)), 61.12 (CH_2-5′), 68.70 (CH-4′), 118.23 (C-5), 127.08 (–CH=(N–Bn)), 128.40 (2×–CH=(N–Bn)), 128.59 (2×–CH=(N–Bn)), 138.93 (>C=(N–Bn)), 141.07 (C-8), 150.13 (C-4), 152.24 (C-2), 155.98 (C-6).

4.1.17. (3*R*,4*R*)-4-(Adenin-9-yl)-1-*N*-benzyl-3-dimethoxytrityloxypyrrolidine (11), (3*S*,4*R*)-4-(adenin-9-yl)-1-*N*-benzyl-3-dimethoxytrityloxypyrrolidine (12), and (3*R*,4*R*)-4-adenin-7-yl-1-*N*-benzyl-3-dimethoxytrityloxypyrrolidine (13). A mixture of **10** (7 g, 12.2 mmol) and adenine (3.3 g, 25 mmol) was co-evaporated with toluene (2×20 mL), DMF (2×20 mL), and suspended in DMF (120 mL). Cs_2CO_3 (8 g, 25 mmol) was added, and the reaction mixture was stirred at 110 °C for 4 d. DMF was evaporated and the crude material was purified on a silica gel column using a linear gradient of ethyl acetate in toluene followed by a linear gradient of ethanol in ethyl acetate. Compounds **11**, **12**, and **13** were obtained in 33% (2.5 g), 11% (0.8 g), and 10% yield (0.7 g), respectively, in the form of yellowish foams.

Compound **11**. HRMS for $C_{37}H_{36}N_6O_3$ $(M+H)^+$ calcd: 613.2927, found: 613.2872. RP-HPLC t_R=9.77 min (A to C/20 min). ^1H NMR (500 MHz, DMSO-d_6): 1.91 (dd, 1H, J_{gem}=9.3, $J_{2'b,3'}$=4.6, H-2′b), 2.25 (dd, 1H, J_{gem}=9.3, $J_{2'a,3'}$=6.9, H-2′a), 2.66 (dd, 1H, J_{gem}=9.4, $J_{5'b,4'}$=6.0, H-5′b), 2.92 (dd, 1H, J_{gem}=9.4, $J_{5'a,4'}$=7.3, H-5′a), 3.43 and 3.47 (2×d, 2H, J_{gem}=12.9, CH_2-Ph), 3.66 and 3.63 (2×s, 6H, CH_3O-DMTr), 4.47 (m, 1H, $J_{3',2'}$=6.9, 4.6, $J_{3',4'}$=4.2, H-3′), 5.07 (m, 1H, $J_{4',5'}$=7.3, 6.0, $J_{4',3'}$=4.2, H-4′), 6.57 (m, 2H, H-*m*-C_6H_4-DMTr), 6.68 (m, 2H, H-*m*-C_6H_4-DMTr), 7.08 (m, 2H, H-*o*-C_6H_4-DMTr), ~7.12 (br s, 2H, NH_2), 7.12 (m, 7H, H-*o*-C_6H_4-DMTr+H-Bn), 7.18 (m, 2H, H-*m*-C_6H_5-DMTr), 7.21 (m, 1H, H-*p*-C_6H_5-DMTr), 7.26 (m, 2H, H-*o*-C_6H_5-DMTr), 8.09 (s, 1H, H-2), 8.13 (s, 1H, H-8). ^{13}C NMR (125.8 MHz, DMSO-d_6): 55.04 and 55.12 (CH_3O-DMTr), 57.32 (CH_2-2′), 58.99 (CH_2-5′), 59.56 (CH_2-Ph), 60.56 (CH-3′), 78.06 (CH-4′), 86.50 (C-DMTr), 113.08 and 113.23 (CH-*m*-C_6H_4-DMTr), 118.95 (C-5), 126.78 (CH-*p*-Bn), 127.07 (CH-*p*-C_6H_5-DMTr), 127.62 (CH-*o*-Bn), 127.84 (CH-*m*-Bn), 128.26 and 128.67 (CH-*o*,*m*-C_6H_5-DMTr), 129.65 and 129.86 (CH-*m*-C_6H_4-DMTr), 135.82 and 135.93 (C-*i*-C_6H_4-DMTr), 138.22 (C-*i*-Bn), 139.78 (CH-8), 145.43 (C-*i*-C_6H_5-DMTr), 149.48 (C-4),

152.47 (CH-2), 156.22 (C-6), 158.16 and 158.30 (C-*p*-C_6H_4-DMTr).

Compound **12**. HRMS for $C_{37}H_{36}N_6O_3$ $(M+H)^+$ calcd: 613.2927, found: 613.2909. RP-HPLC t_R=9.31 min (A to C/20 min). ^{1}H NMR (500 MHz, DMSO-d_6): 1.98 (dd, 1H, J_{gem}=10.4, $J_{2'b,3'}$=7.5, H-2′b), 2.16 (dd, 1H, J_{gem}=10.4, $J_{2'a,3'}$=4.9, H-2′a), 2.78 (dd, 1H, J_{gem}=10.2, $J_{5'b,4'}$=5.9, H-5′b), 2.87 (dd, 1H, J_{gem}=10.2, $J_{5'a,4'}$=3.8, H-5′a), 3.46 and 3.50 (2×d, 2H, J_{gem}=12.8, CH_2-Ph), 3.70 (s, 6H, CH_3O-DMTr), 4.37 (dt, 1H, $J_{3',2'}$=7.5, 4.9, $J_{3',4'}$=7.5, H-3′), 5.06 (ddd, 1H, $J_{4',3'}$=7.5, $J_{4',5'}$=5.9, 3.8, H-4′), 6.70 (m, 4H, H-*m*-C_6H_4-DMTr), 6.94 (m, 2H, H-*o*-C_6H_4-DMTr), 6.96 (m, 2H, H-*o*-C_6H_4-DMTr), 7.04 (m, 2H, H-Bn), 7.12 (br s, 2H, NH_2), 7.13 (m, 3H, H-Bn), 7.18 (m, 2H, H-*m*-C_6H_5-DMTr), 7.21 (m, 1H, H-*p*-C_6H_5-DMTr), 7.27 (m, 2H, H-*o*-C_6H_5-DMTr), 8.17 (s, 1H, H-2), 8.39 (s, 1H, H-8). ^{13}C NMR (125.8 MHz, DMSO-d_6): 54.30 (CH-3′), 55.16 (CH_3O-DMTr), 57.56 (CH_2-2′), 58.54 (CH_2-5′), 58.98 (CH_2-Ph), 71.79 (CH-4′), 86.81 (C-DMTr), 113.27 (CH-*m*-C_6H_4-DMTr), 118.26 (C-5), 126.80 (CH-*p*-Bn), 127.08 (CH-*p*-C_6H_5-DMTr), 127.61 (CH-*o*-Bn), 127.91 (CH-*m*-Bn), 128.33 (CH-*o*,*m*-C_6H_5-DMTr), 128.59 (CH-*o*-C_6H_4-DMTr), 129.76 (CH-C_6H_5-DMTr), 135.69 and 136.01 (C-*i*-C_6H_4-DMTr), 138.54 (C-*i*-Bn), 141.29 (CH-8), 145.26 (C-*i*-C_6H_5-DMTr), 150.38 (C-4), 152.37 (CH-2), 156.20 (C-6), 158.32 (C-*p*-C_6H_4-DMTr).

Compound **13**. HRMS for $C_{37}H_{36}N_6O_3$ $(M+H)^+$ calcd: 613.2927, found: 613.2876. RP-HPLC t_R=8.21 min (A to C/20 min). ^{1}H NMR (500 MHz, DMSO-d_6): 2.01 (dd, 1H, J_{gem}=10.0, $J_{2'b,3'}$=3.8, H-2′b), 2.31 (dd, 1H, J_{gem}=10.0, $J_{2'a,3'}$=6.9, H-2′a), 2.81 (dd, 1H, J_{gem}=9.8, $J_{5'b,4'}$=7.8, H-5′b), 2.96 (dd, 1H, J_{gem}=9.8, $J_{5'a,4'}$=7.4, H-5′a), 3.43 and 3.46 (2×d, 2H, J_{gem}=12.8, CH_2-Ph), 3.63 and 3.66 (2×s, 6H, CH_3O-DMTr), 4.74 (ddd, 1H, $J_{3',2'a}$=6.9, $J_{3',2'b}$=3.8, $J_{3',4'}$=4.2, H-3′), 5.24 (ddd, 1H, $J_{4',3'}$=4.2, $J_{4',5'a}$=7.4, $J_{4',5'b}$=7.2, H-4′), 6.54 and 6.66 (2×m, 4H, H-*m*-C_6H_4-DMTr), 7.06 and 7.10 (2×m, 4H, H-*o*-C_6H_4-DMTr), 7.10 (m, 2H, H-*o*-Bn), 7.18–7.28 (m, 8H, H-*m*-Bn, H-*p*-Bn, C_6H_5-DMTr), 7.71 (s, 1H, H-2), 7.85 and 7.96 (2×br s, 2H, NH_2), 8.34 (s, 1H, H-8). ^{13}C NMR (125.8 MHz, DMSO-d_6): 55.06 and 55.13 (CH_3O-DMTr), 56.41 (CH_2-2′), 59.03 (CH_2-Ph), 59.69 (CH_2-5′), 66.89 (CH-3′), 76.88 (CH-4′), 86.52 (C-DMTr), 113.04 and 113.19 (CH-*m*-C_6H_4-DMTr), 120.76 (C-5), 126.78 (CH-*p*-Bn), 127.09 (CH-*p*-C_6H_5-DMTr), 127.63 (CH-*o*-Bn), 127.81 (CH-*m*-Bn), 128.28 and 128.70 (CH-*o*-C_6H_4-DMTr), 129.67 (CH-*o*-C_6H_5-DMTr), 129.88 (CH-*m*-C_6H_5-DMTr), 135.86 and 135.89 (C-*i*-C_6H_4-DMTr), 138.27 (C-*i*-Bn), 142.60 (CH-8), 145.47 (C-*i*-C_6H_5-DMTr), 149.44 (C-4), 152.54 (CH-2), 154.86 (C-6), 158.15 and 158.29 (C-*p*-C_6H_4-DMTr).

4.1.18. (3*R*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-3,4-dihydroxypyrrolidine (14a). *tert*-Butyloxycarbonyl anhydride (15 g, 70 mmol) was added dropwise to the vigorously stirred mixture of **8a** (4.8 g, 46.6 mmol) and sodium hydrogen carbonate (34 g, 400 mmol) in 50% aqueous dioxane (400 mL). The reaction mixture was stirred at rt for 2 h. The suspension was filtered and the filtrate was concentrated in vacuo. Pure compound **14a** was obtained by chromatography on silica gel using a linear gradient of ethanol in chloroform in 89% yield (8.4 g) as a white solid (NMR: 1:1 ratio of amide isomers).

HRMS for $C_9H_{17}NO_4$ $(M+H)^+$ calcd: 204.1236 found: 204.1245. ^{1}H NMR (400 MHz, DMSO-d_6): 1.39 (s, 9H, CH_3-^{t}Bu), 3.11 (dd, 2H, J_{gem}=11.4, J=3.5, CH_bH$_a$-N), 3.32+3.36 (dd, 2H, J_{gem}=11.4, J=3.9, CH_aH$_b$-N), 3.86 (br q, 2H, J=3.9, 3.5, $J_{CH,OH}$=3.2, CHO), 5.06 (d, 2H, $J_{OH,CH}$=3.2, OH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 28.42 (CH_3-^{t}Bu), 51.86+52.16 (CH_2N), 73.85+74.67 (CHO), 78.28 (C-^{t}Bu), 154.13 (CO).

4.1.19. (3*S*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-3,4-dihydroxypyrrolidine (14b). Desired compound **14b** was prepared from **8b** (5 g, 48.5 mmol) using the same procedure as for **14a** in 85% yield (8.3 g) in the form of a white solid.

^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **14a**.

4.1.20. (3*R*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-hydroxypyrrolidine (15a). Dimethoxytrityl chloride (35.4 g, 105 mmol) was added to the solution of **14a** (19.3 g, 95 mmol) in pyridine (1 L). The reaction mixture was stirred at rt for 5 d, quenched with methanol (5 mL), and concentrated in vacuo. Pure compound **15a** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 75% yield (36.2 g) in the form of a yellowish foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{30}H_{35}NO_6$ $(M+H)^+$ calcd: 506.2543, found: 506.2506. ^{1}H NMR (500 MHz, DMSO-d_6): 1.29+1.37 (s, 9H, CH_3-^{t}Bu), 2.31+2.52 (dd, 1H, J_{gem}=11.8, $J_{2b,3}$=2.0, H-2b), 2.66+2.82 (dd, 1H, J_{gem}=11.8, $J_{2a,3}$=4.6, H-2a), 3.42 (m, 2H, H-5), 3.74+3.735 (s, 6H, CH_3O-DMTr), 3.79+3.81 (m, 1H, $J_{3,2}$=4.6, 2.0, $J_{3,4}$=2.4, H-3), 3.88+3.77 (m, 1H, $J_{4,OH}$=3.8, $J_{4,3}$=2.4, H-4), 5.14+5.06 (d, 1H, $J_{OH,4}$=3.8, OH-4), 6.90 (m, 4H, H-*m*-C_6H_4-DMTr), 7.14–7.41 (m, 9H, H-*o*-C_6H_4-DMTr+H-C_6H_5-DMTr). ^{13}C NMR (125.8 MHz, DMSO-d_6): 28.17+28.30 (CH_3-^{t}Bu), 49.84+50.07 (CH_2-5), 51.51+52.13 (CH_2-2), 55.19 (CH_3O-DMTr), 72.98+73.58 (CH-4), 77.31+76.75 (CH-3), 78.11+78.36 (C-^{t}Bu), 86.27+86.37 (C-DMTr), 113.44+113.47 (CH-*m*-C_6H_4-DMTr), 126.89+126.94 (CH-*p*-C_6H_5-DMTr), 127.86+127.94 (CH-*m*-C_6H_5-DMTr), 128.02 (CH-*o*-C_6H_5-DMTr), 129.94+129.98 (CH-*o*-C_6H_4-DMTr), 136.07+136.21 (C-*i*-C_6H_4-DMTr), 136.37+136.26 (C-*i*-C_6H_4-DMTr), 145.50 (C-*i*-C_6H_5-DMTr), 153.91+153.88 (CO), 158.44+158.37 (C-*p*-C_6H_4-DMTr).

4.1.21. (3*S*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-hydroxypyrrolidine (15b). Desired compound **15b** was prepared from **14b** (39.2 g, 193 mmol) using the same procedure as for **15a** in 80% yield (77.8 g) in the form of a yellowish foam.

HRMS for $C_{30}H_{35}NO_6$ $(M+H)^+$ calcd: 506.2543, found: 506.2506. ^{1}H NMR, ^{13}C NMR spectra were identical to those of compound **15a**.

4.1.22. (3*R*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-4-dimethoxytrityloxy-3-mesyloxypyrrolidine (16a). Mesyl chloride (1.6 mL, 20 mL) was added dropwise to the solution of **15a** (2 g, 4 mmol) and DMAP (2.4 g, 20 mmol) in DCM

(30 mL) at 0 °C. The reaction mixture was stirred at rt for 1 h, cooled to 0 °C, and quenched with water (3 mL). The solution was washed with a saturated solution of sodium hydrogen carbonate, and the organic layer was dried over sodium sulfate. Solvents were evaporated and pure compound **16a** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 83% yield (1.93 g) in the form of a yellowish foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{31}H_{37}NO_8S$ $(M+H)^+$ calcd: 584.2318, found: 584.2379. ^{1}H NMR (400 MHz, DMSO-d_6): 1.29+1.38 (s, 9H, CH_3-^{t}Bu), 2.29+2.57 (br dd, 1H, J_{gem}=12.3, $J_{5b,4}$=2.2, H-5b), 2.63+2.83 (dd, 1H, J_{gem}=12.3, $J_{5a,4}$=4.5, H-5a), 3.23+3.18 (s, 3H, CH_3S), 3.39+3.46 (br dd, 1H, J_{gem}=12.7, $J_{2b,3}$=1.8, H-2b), 3.70 (br d, 1H, J_{gem}=12.7, H-2a), 3.74 (s, 6H, CH_3O-DMTr), 4.15 (m, 1H, H-4), 5.11+4.93 (br dt, 1H, $J_{3,4}$=4.5, $J_{3,2}$=1.8, H-3), 6.90 (m, 4H, H-*m*-C_6H_4-DMTr), 7.16 (m, 1H, H-*p*-C_6H_5-DMTr), 7.22–7.36 (m, 6H, H-*m*-C_6H_5-DMTr and H-*o*-C_6H_4-DMTr), 7.44+7.41 (m, 2H, H-*o*-C_6H_5-DMTr). ^{13}C NMR (100.6 MHz, DMSO-d_6): 28.06+28.20 (CH_3-^{t}Bu), 37.77 (CH_3-S), 49.38+49.76 (CH_2-5), 49.51+49.90 (CH_2-2), 55.24 (CH_3O-DMTr), 75.16+74.53 (CH-4), 78.84+79.19 (C-^{t}Bu), 81.57+82.12 (CH-3), 87.08+7.15 (C-DMTr), 113.62+113.66 (CH-*m*-C_6H_4-DMTr), 127.13 (CH-*p*-C_6H_5-DMTr), 127.79+127.89 (CH-*m*-C_6H_5-DMTr), 128.20 (CH-*o*-C_6H_5-DMTr), 129.96+130.07 (CH-*o*-C_6H_4-DMTr), 135.37+135.54 (C-*i*-C_6H_4-DMTr), 135.81+135.66 (C-*i*-C_6H_4-DMTr), 144.96 (C-*i*-C_6H_5-DMTr), 153.62+153.48 (CO), 158.64+158.58 (C-*p*-C_6H_4-DMTr).

4.1.23. (3*S*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-4-dimethoxytrityloxy-3-mesyloxypyrrolidine (16b). Desired compound **16b** was prepared from **15b** (7.2 g, 14.2 mmol) using the same procedure as for **16b** in 90% yield (7.5 g) in the form of a yellowish foam.

^{1}H NMR, ^{13}C NMR and HRMS spectra were identical to those of compound **16a**.

4.1.24. (3*R*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-(thymin-1-yl)-pyrrolidine (17a). Compound **17a** was prepared from **16a** (1.8 g, 3.1 mmol) and thymine (0.8 g, 6 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 33% yield (0.6 g) in the form of a yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{35}H_{39}N_3O_7$ $(M+H)^+$ calcd: 614.2866, found: 614.2884. ^{1}H NMR (500 MHz, DMSO-d_6): 1.27+1.36 (br s, 9H, CH_3-^{t}Bu), 1.83 (br s, 3H, CH_3-5), 2.11+2.43 (br dd, 1H, J_{gem}=11.4, $J_{2'b,3'}$=4.1, H-2′b), 2.43+2.69 (br dd, 1H, J_{gem}=11.4, $J_{2'a,3'}$=5.9, H-2′a), 3.58+3.66 (br m, 1H, H-5′b), 3.68+3.66 (br m, 1H, H-5′a), 3.74 (s, 6H, CH_3O-DMTr), 4.13+4.25 (br m, 1H, H-3′), 5.16+5.03 (br m, 1H, H-4′), 6.86 (m, 4H, H-*m*-C_6H_4-DMTr), 7.11–7.36 (m, 4H, H-*o*-C_6H_4-DMTr+C_6H_5-DMTr), 7.63+7.57 (br s, 1H, H-6), 11.49+11.44 (br s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 12.22 (CH_3-5), 28.05+28.22 (CH_3-^{t}Bu), 46.06+46.93 (CH_2-5′), 50.03+50.24 (CH_2-2′), 54.68 (CH-4′), 55.22+55.24 (CH_3O-DMTr), 71.40+70.78 (CH-3′), 78.69+79.03 (C-^{t}Bu), 87.01 (C-DMTr), 108.85+108.71 (C-5), 113.43+113.48 (CH-*m*-C_6H_4-DMTr), 126.96, 127.70, and 128.04 (CH-C_6H_5-DMTr), 130.03 (CH-*o*-C_6H_4-DMTr), 135.44 and 136.01+135.64 and 135.89 (C-*i*-C_6H_4-DMTr), 139.16+139.44 (CH-6), 145.26 (C-*i*-C_6H_5-DMTr), 151.69+151.55 (C-2), 153.46+153.14 (CO), 158.55 (C-*p*-C_6H_4-DMTr), 163.86 (C-4).

4.1.25. (3*R*,4*S*)-4-(Adenin-9-yl)-1-*N*-*tert*-butyloxycarbonyl-3-dimethoxytrityloxypyrrolidine (17b). Compound **17b** was prepared from **16a** (5.2 g, 9 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 55% yield (3.1 g) in the form of a yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{35}H_{38}N_6O_5$ $(M+H)^+$ calcd: 623.2982, found: 623.3004. ^{1}H NMR (400 MHz, DMSO-d_6): 1.29+1.36 (s, 9H, CH_3-^{t}Bu), 2.31+2.58 (dd, 1H, J_{gem}=11.8, $J_{2'b,3'}$=4.7, H-2′b), 2.53+2.73 (dd, 1H, J_{gem}=11.8, $J_{2'a,3'}$=6.5, H-2′a), 3.70 (s, 6H, CH_3O-DMTr), 3.77 (m, 1H, H-5′b), 3.97+3.91 (dd, 1H, J_{gem}=11.3, $J_{5'a,4'}$=6.0, H-5′a), 4.22 (q, 1H, $J_{3',2'}$=6.5, 4.7, $J_{3',2'}$=6.0, H-3′), 5.21 (q, 1H, $J_{4',3'}$=$J_{4',5'}$=6.0, H-4′), 6.67–6.78 (m, 4H, H-*m*-C_6H_4-DMTr), 6.93–7.00 (m, 4H, H-*o*-C_6H_4-DMTr), 7.03–7.18 (m, 5H, C_6H_5-DMTr), 7.33 (br s, 2H, NH_2), 8.16+8.14 (s, 1H, H-2), 8.35+8.24 (s, 1H, H-8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 28.10+28.24 (CH_3-^{t}Bu), 47.06+48.15 (CH_2-5′), 49.68 (CH_2-2′), 54.50+55.04 (CH-4′), 55.17 (CH_3O-DMTr), 71.65+71.13 (CH-3′), 78.70+79.05 (C-^{t}Bu), 86.59 (C-DMTr), 113.32 (CH-*m*-C_6H_4-DMTr), 118.81 (C-5), 126.84, 127.58, 127.73, and 127.91 (CH-C_6H_5-DMTr), 129.93 (CH-*o*-C_6H_4-DMTr), 135.37, 135.82+135.64, and 135.71 (C-*i*-C_6H_4-DMTr), 139.91 (CH-8), 145.09 (C-*i*-C_6H_5-DMTr), 150.61+150.50 (C-4), 152.68 (CH-2), 153.55+153.31 (CO), 156.34 (C-6), 158.43+158.49 (C-*p*-C_6H_4-DMTr).

4.1.26. (3*S*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-(thymin-1-yl)-pyrrolidine (17c). Compound **17c** was prepared from **16b** (4.5 g, 7.8 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 35% yield (1.7 g) in the form of a yellow foam.

HRMS for $C_{35}H_{39}N_3O_7$ $(M+H)^+$ calcd: 614.2866, found: 614.2868. ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **17a**.

4.1.27. (3*S*,4*R*)-4-(Adenin-9-yl)-1-*N*-*tert*-butyloxycarbonyl-3-dimethoxytrityloxypyrrolidine (17d). Compound **17d** was prepared from **16b** (6.9 g, 12 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 54% yield (4 g) in the form of a yellow foam.

HRMS for $C_{35}H_{38}N_6O_5$ $(M+H)^+$ calcd: 623.2982, found: 623.2983. ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **17b**.

4.1.28. (3*S*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-(4-nitro-benzoyloxy)-pyrrolidine (18a). A mixture of **15a** (39 g, 77 mmol), 2,6-lutidine (26.8 mL,

231 mmol), *p*-nitrobenzoic acid (19.4 g, 115.5 mmol), and triphenylphosphine (60.6 g, 231 mmol) was co-evaporated with THF (2×200 mL), dissolved in the same solvent (770 mL), and DIAD (44.8 mL, 231 mmol) was added at 0 °C under argon atmosphere. The reaction mixture was stirred at rt for 3 h, concentrated in vacuo, and compound **18a** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 88% yield (44.3 g) in the form of a yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{37}H_{39}N_2O_9$ $(M+H)^+$ calcd: 677.2475, found: 677.2452. ^{1}H NMR (500 MHz, DMSO-d_6): 1.30+1.32 (s, 9H, CH_3-^{t}Bu), 2.27+2.73 (dd, 1H, J_{gem}=10.5, $J_{2b,3}$=7.3, H-2b), 2.72+2.93 (t, 1H, J_{gem}=10.5, $J_{2a,3}$=9.0, H-2a), 3.38+3.39 (br d, 1H, J_{gem}=13.0, $J_{5b,4}\leq$2, H-5b), 3.47+3.45 (dd, 1H, J_{gem}=13.0, $J_{5a,4}$=4.0, H-5a), 3.71 (s, 3H, CH_3O-DMTr), 3.72 (s, 3H, CH_3O-DMTr), 4.18+4.28 (dt, 1H, $J_{3,2}$=9.0, 7.3, $J_{3,4}$=4.0, H-3), 5.49+5.13 (br t, 1H, $J_{4,5}$=4.0, ≤2, $J_{4,3}$=4.0, H-4), 6.82–6.87 (m, 4H, H-*m*-C_6H_4-DMTr), 7.12–7.25 (m, 5H, H-C_6H_5-DMTr), 7.30–7.40 (m, 4H, H-*o*-C_6H_4-DMTr), 8.32+8.30 (m, 2H, C_6H_4-NO_2), 8.41 (m, 2H, C_6H_4-NO_2). ^{13}C NMR (125.8 MHz, DMSO-d_6): 28.05+28.13 (CH_3-^{t}Bu), 47.90+47.68 (CH_2-2), 48.52+49.31 (CH_2-5), 55.14+55.17 (CH_3O-DMTr), 71.90+71.36 (CH-3), 74.06+74.56 (CH-4), 78.86+78.83 (C-^{t}Bu), 86.74+86.61 (C-DMTr), 113.50+113.40 (CH-*m*-C_6H_4-DMTr), 124.10 (CH-$C_6H_4NO_2$), 126.89+127.00 (CH-*p*-C_6H_5-DMTr), 127.47+127.65 (CH-*m*-C_6H_5-DMTr), 128.01 (CH-*o*-C_6H_5-DMTr), 129.84+129.97 (CH-*o*-C_6H_5-DMTr), 130.98 (CH-$C_6H_4NO_2$), 135.37 (C-*i*-$C_6H_4NO_2$), 135.37+135.57 (C-*i*-C_6H_4-DMTr), 136.01+135.90 (C-*i*-C_6H_4-DMTr), 145.27+145.19 (C-*i*-C_6H_5-DMTr), 150.57 (C-*i*-$C_6H_4NO_2$), 153.50+153.27 (CO-carbamate), 158.61+158.50 (C-*p*-C_6H_4-DMTr), 164.12+164.00 (CO-ester).

4.1.29. (3*R*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-(4-nitro-benzoyloxy)-pyrrolidine (18b). Compound **18b** was prepared from **15a** (7.6 g, 15 mmol) using the same procedure as for the compound **18a** in 97% yield (9.5 g) in the form of a yellow foam.

^{1}H NMR, ^{13}C NMR and HRMS spectra were identical to those of compound **18a**.

4.1.30. (3*R*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-hydroxypyrrolidine (19a). Gaseous ammonia was introduced into the solution of **18a** (44.3 g, 67.7 mmol) in methanol (200 mL) at 0 °C over a period of 20 min. The solution was set aside at rt for 1 h, methanol was evaporated, and the residue was treated with a mixture of petroleum ether/toluene (1:1, 500 mL). 4-Nitrobenzoic amide was filtered off and the filtrate was concentrated in vacuo. Desired product **19a** was obtained by chromatography on silica gel using a linear gradient of toluene in petroleum ether followed by a linear gradient of ethyl acetate in toluene in 90% yield (30.8 g) in the form of yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{30}H_{35}NO_6$ $(M+H)^+$ calcd: 505.2464, found: 505.2456. ^{1}H NMR (500 MHz, DMSO-d_6): 1.27+1.32 (s, 9H, CH_3-^{t}Bu), 2.20+2.46 (dd, 1H, J_{gem}=10.6, $J_{2b,3}$=6.7, H-2b), 2.49+2.68 (dd, 1H, J_{gem}=10.6, $J_{2a,3}$=7.3, H-2a), ~3.10 (m, 2H, H-5), 3.735+3.73 (s, 6H, CH_3O-DMTr), 3.78+3.85 (m, 1H, $J_{3,2}$=7.3, 6.7, $J_{3,4}$=3.8, H-3), 3.88+3.69 (m, 1H, $J_{4,OH}$=4.0, $J_{4,3}$=3.8, H-4), 5.03+4.93 (d, 1H, $J_{OH,4}$=4.0, OH-4), 6.89+6.90 (m, 4H, H-*m*-C_6H_4-DMTr), 7.24 (m, 1H, H-*p*-C_6H_5-DMTr), 7.305 (m, 2H, H-*m*-C_6H_5-DMTr), 7.37–7.41 (m, 4H, H-*o*-C_6H_4-DMTr), 7.545+7.51 (m, 2H, H-*o*-C_6H_5-DMTr). ^{13}C NMR (125.8 MHz, DMSO-d_6): 28.11+28.22 (CH_3-^{t}Bu), 47.50+47.27 (CH_2-5), 50.86+51.61 (CH_2-2), 55.16 (CH_3O-DMTr), 69.41+69.83 (CH-4), 73.44+72.98 (CH-3), 78.05+78.28 (C-^{t}Bu), 86.23 (C-DMTr)), 113.32+113.39 (CH-*m*-C_6H_4-DMTr), 126.74+126.82 (CH-*p*-C_6H_5-DMTr), 127.86+127.97 (CH-*m*-C_6H_5-DMTr), 127.92 (CH-*o*-C_6H_5-DMTr), 129.99+130.05 (CH-*o*-C_6H_4-DMTr), 136.23+136.43 (C-*i*-C_6H_4-DMTr), 136.72+136.59 (C-*i*-C_6H_4-DMTr), 145.79 +145.73 (C-*i*-C_6H_5-DMTr), 153.67+153.50 (CO), 158.42+158.32 (C-*p*-C_6H_4-DMTr).

4.1.31. (3*S*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-hydroxypyrrolidine (19b). Compound **19b** was prepared from **18b** (9.5 g, 14.6 mmol) using the same procedure as for the compound **19a** in 97% yield (7.2 g) in the form of a yellow foam.

^{1}H NMR, ^{13}C NMR and HRMS spectra were identical to those of compound **19a**.

4.1.32. (3*S*,4*R*)-1-*N*-*tert*-Butyloxycarbonyl-4-dimethoxytrityloxy-3-mesyloxypyrrolidine (20a). Compound **20a** was prepared from **19a** (30.8 g, 61 mmol) using the same procedure as for the compound **16a** in 85% yield (30.2 g) in the form of a yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{31}H_{37}NO_8S$ $(M+H)^+$ calcd: 584.2318, found: 584.2379. ^{1}H NMR (500 MHz, DMSO-d_6): 1.26+1.32 (s, 9H, CH_3-^{t}Bu), 1.95+2.32 (dd, 1H, J_{gem}=10.7, $J_{5b,4}$=7.4, H-5b), 2.37+2.52 (dd, 1H, J_{gem}=10.7, $J_{5a,4}$=9.2, H-5a), 3.31 (s, 3H, CH_3S), ~3.38 (m, 2H, H-2), 3.75 (s, 6H, CH_3O-DMTr), 4.06+4.18 (ddd, 1H, $J_{4,5}$=9.2, 7.4, $J_{4,3a}$=3.9, H-4), 5.17+4.96 (m, 1H, $J_{3,4}$=3.9, H-3), 6.905+6.92 (m, 4H, H-*m*-C_6H_4-DMTr), 7.25+7.23 (m, 1H, H-*p*-C_6H_5-DMTr), 7.32 (m, 2H, H-*m*-C_6H_5-DMTr), 7.34+7.37 (m, 4H, H-*o*-C_6H_4-DMTr), 7.50+7.46 (m, 2H, H-*o*-C_6H_5-DMTr). ^{13}C NMR (125.8 MHz, DMSO-d_6): 27.99+28.13 (CH_3-^{t}Bu), 38.47 (CH_3-S), 47.34+47.12 (CH_2-5), 48.96+49.66 (CH_2-2), 55.17 (CH_3O-DMTr), 71.32+70.90 (CH-4), 78.65+78.96 (C-^{t}Bu), 80.23+80.76 (CH-3), 86.90+86.88 (C-DMTr), 113.57+113.38 (CH-*m*-C_6H_4-DMTr), 126.92+127.04 (CH-*p*-C_6H_5-DMTr), 127.70+127.91 (CH-*m*-C_6H_5-DMTr), 128.09 (CH-*o*-C_6H_5-DMTr), 129.91+130.01 (CH-*o*-C_6H_4-DMTr), 135.53+135.78 (C-*i*-C_6H_4-DMTr), 136.20+136.04 (C-*i*-C_6H_4-DMTr), 145.20+145.08 (C-*i*-C_6H_5-DMTr), 153.33+153.11 (CO), 158.56+158.45 (C-*p*-C_6H_4-DMTr).

4.1.33. (3*R*,4*S*)-1-*N*-*tert*-Butyloxycarbonyl-4-dimethoxytrityloxy-3-mesyloxypyrrolidine (20b). Compound **20b** was prepared from **19b** (7.2 g, 14.2 mmol) using the same procedure as for the compound **16a** in 90% yield (7.5 g) in the form of a yellow foam.

^{1}H NMR, ^{13}C NMR and HRMS spectra were identical to those of compound **20a**.

4.1.34. (3*R*,4*R*)-*N*-*tert*-Butyloxycarbonyl-3-dimethoxytrityloxy-4-(thymin-1-yl)-1-pyrrolidine (21a). Compound **21a** was prepared from **20a** (8.7 g, 15 mmol) and thymine (3.8 g, 30 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 33% yield (0.6 g) in the form of a yellow foam.

HRMS for $C_{35}H_{39}N_3O_7$ $(M+H)^+$ calcd: 614.2866, found: 614.2876. ^{1}H NMR (400 MHz, DMSO-d_6): 1.26+1.32 (br s, 9H, CH_3-^{t}Bu), 1.73+1.69 (br s, 3H, CH_3-5), 2.15+2.46 (dd, 1H, J_{gem}=11.4, $J_{2'b,3'}$=7.1, H-2′b), 2.33+2.51 (dd, 1H, J_{gem}=11.4, $J_{2'a,3'}$=7.7, H-2′a), 3.15 (br t, 1H, J_{gem}=$J_{5'b,4'}$=10.2, H-5′b), 3.50+3.56 (br t, 1H, J_{gem}=$J_{5'a,4'}$=10.2, H-5′a), 3.73+3.72 (s, 6H, CH_3O-DMTr), 4.27+4.39 (br q, 1H, $J_{3',2'}$=7.7, 7.1, $J_{3',4'}$=8.0, H-3′), 5.14 (br q, 1H, $J_{4',5'}$=10.2, $J_{4',3'}$=8.0, H-4′), 6.79 and 6.85 (2×m, 4H, H-*m*-C_6H_4-DMTr), 7.13–7.27 (m, 7H, H-*o*-C_6H_4-DMTr, H-*m*,*p*-C_6H_5-DMTr), 7.30–7.38 (m, 2H, H-*o*-C_6H_5-DMTr), 7.59+7.49 (br s, 1H, H-6), 11.43+11.37 (br s, 1H, NH). ^{13}C NMR (100.6 MHz, DMSO-d_6): 12.18 (CH_3-5), 28.05+28.17 (CH_3-^{t}Bu), 44.53+45.39 (CH_2-5′), 49.64+49.46 (CH_2-2′), 55.17 (CH_3O-DMTr), 59.43+59.94 (CH-4′), 73.49+73.84 (CH-3′), 78.62+78.95 (C-^{t}Bu), 85.95 (C-DMTr), 110.03+109.93 (C-5), 113.37 (CH-*m*-C_6H_4-DMTr), 126.96, 127.57, and 127.99 (CH-C_6H_5-DMTr), 130.06 (CH-*o*-C_6H_4-DMTr), 135.45, 136.04+135.79, and 135.88 (C-*i*-C_6H_4-DMTr), 138.99+138.76 (CH-6), 145.34 (C-*i*-C_6H_5-DMTr), 151.35 (C-2), 153.19+153.10 (CO), 158.57+158.46(C-*p*-C_6H_4-DMTr), 163.99 (C-4).

4.1.35. (3*R*,4*R*)-4-(Adenin-9-yl)-1-*N*-*tert*-butyloxycarbonyl-3-dimethoxytrityloxypyrrolidine (21b). Compound **21b** was prepared from **20a** (16 g, 27.5 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 46% yield (8.4 g) in the form of a yellow foam (NMR: 3:2 ratio of amide isomers; separated signals are arranged: major+minor).

HRMS for $C_{35}H_{38}N_6O_5$ $(M+H)^+$ calcd: 623.2982, found: 623.2995. ^{1}H NMR (400 MHz, DMSO-d_6): 1.28+1.32 (s, 9H, CH_3-^{t}Bu), 2.15+2.46 (dd, 1H, J_{gem}=11.8, $J_{2'b,3'}$=7.1, H-2′b), 2.35+2.55 (dd, 1H, J_{gem}=11.8, $J_{2'a,3'}$=7.6, H-2′a), 3.66–3.78 (m, 2H, H-5′), 3.71+3.67 (s, 6H, CH_3O-DMTr), 4.50+4.64 (br q, 1H, $J_{3',2'}$=7.6, 7.1, $J_{3',4'}$=7.1, H-3′), 5.25 (br q, 1H, $J_{4',5'}$=9.2, $J_{4',3'}$=7.1, H-4′), 6.61 and 6.79 (2×m, 4H, H-*m*-C_6H_4-DMTr), 6.90 and 7.10 (2×m, 4H, H-*o*-C_6H_4-DMTr), 7.10–7.15 (m, 5H, C_6H_5-DMTr), 7.38+7.35 (br s, 2H, NH_2), 8.07+8.05 (s, 1H, H-2), 8.36+8.28 (s, 1H, H-8). ^{13}C NMR (100.6 MHz, DMSO-d_6): 28.04+28.16 (CH_3-^{t}Bu), 44.94+45.89 (CH_2-5′), 49.53+49.33 (CH_2-2′), 55.17+55.08 (CH_3O-DMTr), 58.50+59.05 (CH-4′), 73.89+73.43 (CH-3′), 78.72+79.05 (C-^{t}Bu), 86.03 (C-DMTr), 113.18 and 113.32 (CH-*m*-C_6H_4-DMTr), 119.86 (C-5), 126.79, 127.27, and 127.86 (CH-C_6H_5-DMTr), 129.54, 129.97+129.44, and 129.87 (CH-*o*-C_6H_4-DMTr), 135.24, 135.84+135.57, and 135.67 (C-*i*-C_6H_4-DMTr), 140.87+140.68 (CH-8), 145.27+145.18 (C-*i*-C_6H_5-DMTr), 149.93+149.81 (C-4), 152.54 (CH-2), 153.22+153.07 (CO), 156.46 (C-6), 158.36, 158.61+158.22, and 158.41(C-*p*-C_6H_4-DMTr).

4.1.36. (3*S*,4*S*)-4-(Thymin-1-yl)-1-*N*-*tert*-butyloxycarbonyl-3-dimethoxytrityloxypyrrolidine (21c). Compound **21c** was prepared from **20b** (7.5 g, 13 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 25% yield (2 g) in the form of a yellow foam.

HRMS for $C_{35}H_{39}N_3O_7$ $(M+H)^+$ calcd: 614.2866, found: 614.2874. ^{1}H NMR and ^{13}C NMR spectra were identical to those of compound **21a**.

4.1.37. (3*S*,4*S*)-4-(Adenin-9-yl)-1-*N*-*tert*-butyloxycarbonyl-3-dimethoxytrityloxypyrrolidine (21d). Compound **21a** was prepared from **20b** (7 g, 27.5 mmol) using the same procedure as for the compound **11** (DMSO was used as a solvent instead of DMF) in 51% yield (3.8 g) in the form of a yellow foam.

^{1}H NMR, ^{13}C NMR, and HRMS spectra were identical to those of compound **21b**.

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References and notes

1. Ichikawa, E.; Kato, K. *Curr. Med. Chem.* **2001**, *8*, 385–425.
2. Jeong, L. S.; Shinazi, R. F.; Beach, J. W.; Kim, H. O.; Nampalli, S.; Shanmuganathan, K.; Alves, A. J.; McMillan, A.; Chu, C. K.; Mathis, R. *J. Med. Chem.* **1993**, *36*, 181–195.
3. Kim, H. O.; Ahn, S. K.; Alves, A. J.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Van Roey, P.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.* **1992**, *35*, 1987–1995.
4. Kim, H. O.; Schinazi, R. F.; Shanmuganathan, K.; Jeong, L. S.; Beach, J. W.; Nampalli, S.; Cannon, D. L.; Chu, C. K. *J. Med. Chem.* **1993**, *36*, 519–528.
5. Mansour, T. S.; Jin, H.; Wang, W.; Dixit, D. M.; Evans, C. A.; Tse, H. L. A.; Belleau, B.; Gillard, J. W.; Hooker, E.; Ashman, C.; Cammack, N.; Salomon, H.; Belmonte, A. R.; Wainberg, M. A. *Nucleosides Nucleotides* **1995**, *14*, 627–636.
6. Mao, S.; Bouygues, M.; Welch, C.; Biba, M.; Chilenski, J.; Schinazi, R. F.; Liotta, D. C. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4991–4994.
7. Norbeck, D. W.; Kern, E.; Hayashi, S.; Rosenbrook, W.; Sham, H.; Herrin, T.; Plattner, J. J.; Erickson, J.; Clement, J.; Swanson, R.; Shipkowitz, N.; Hardy, D.; Marsch, K.; Arnett, G.; Shannon, W.; Broder, S.; Mitsuya, H. *J. Med. Chem.* **1990**, *33*, 1281–1285.

8. Hronowski, L. J. J.; Szarek, W. A. *Can. J. Chem.* **1988**, *66*, 61–70.
9. Beres, J.; Sagi, G. Y.; Toemoeskoezi, I.; Gruber, L.; Gulacsi, E.; Oetvoes, L. *Tetrahedron Lett.* **1988**, *22*, 2681–2684.
10. Baumgartner, H.; Bodenteich, M.; Griengl, H. *Tetrahedron Lett.* **1988**, *29*, 5745–5746.
11. Beres, J.; Sagi, G.; Tomoskozi, I.; Gruber, L.; Baitz-Gacs, E.; Otvos, L.; De Clercq, E. *J. Med. Chem.* **1990**, *33*, 1353–1360.
12. Balo, C.; Blanco, J. M.; Fernandez, F.; Lens, E.; Lopez, C. *Tetrahedron* **1998**, *54*, 2833–2842.
13. De Clerq, E. *Int. J. Antimicrob. Agents* **2001**, *18*, 309–328.
14. Kaspersen, F. M.; Bieräugel, H.; Pandit, U. K. *Heterocycles* **1974**, *2*, 15–19.
15. Kaspersen, F. M.; Pandit, U. K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1617–1622.
16. Kaspersen, F. M.; Pandit, U. K. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1798–1802.
17. Ng, K.-M. E.; Orgel, L. E. *J. Med. Chem.* **1989**, *32*, 1754–1757.
18. Peterson, M. L.; Vince, R. *J. Med. Chem.* **1991**, *34*, 2787–2797.
19. Harnden, M. R.; Jarvest, R. L. *Tetrahedron Lett.* **1991**, *32*, 3863–3866.
20. Harnden, M. R.; Jarvest, R. L.; Parratt, M. J. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2259–2263.
21. Westwood, N. B.; Walker, R. T. *Tetrahedron* **1998**, *54*, 13391–13404.
22. Vanek, V.; Budesinsky, M.; Kavenova, I.; Rinnova, M.; Rosenberg, I. *Nucleosides, Nucleotides Nucleic Acids* **2003**, *22*, 1065–1067.
23. Miyabe, H.; Kanehira, S.; Kume, K.; Kandori, H.; Naito, T. *Tetrahedron* **1998**, *54*, 5883–5892.
24. Richichi, B.; Cicchi, S.; Chiacchio, U.; Romeo, G.; Brandi, A. *Tetrahedron Lett.* **2002**, *43*, 4013–4014.
25. Kočalka, P.; Pohl, R.; Rejman, D.; Rosenberg, I. *Nucleosides, Nucleotides Nucleic acids* **2005**, *24*, 805–808.
26. Kočalka, P.; Pohl, R.; Rejman, D.; Rosenberg, I. *Tetrahedron* **2006**, *62*, 5763–5774.
27. Lukes, P.; Pergal, M. *Collect. Czech. Chem. Commun.* **1962**, *27*, 1387–1392.
28. Nemia, M. M. B.; Lee, J.; Joullie, M. M. *Synth. Commun.* **1983**, *13*, 1117–1123.
29. Bhat, K.; Flanagan, D. M.; Joullie, M. M. *Synth. Commun.* **1985**, *15*, 587–598.
30. Nagel, U.; Kinzel, E.; Andrade, J.; Prescher, G. *Chem. Ber.* **1986**, *119*, 3326–3343.
31. Tamazawa, K.; Arima, H.; Koijma, T.; Isomura, Y.; Okada, M.; Fujita, S.; Furuya, T.; Takenaka, T.; Inagaki, O.; Terai, M. *J. Med. Chem.* **1986**, *29*, 2504–2511.
32. Bernardi, A.; Micheli, F.; Potenza, D.; Scolastico, C.; Villa, R. *Tetrahedron Lett.* **1990**, *31*, 4949–4952.
33. Murray, R. W.; Iyanar, K.; Chen, J.; Wearing, J. T. *J. Org. Chem.* **1996**, *61*, 8099–8102.
34. Tomori, H.; Shibutani, K.; Ogura, K. *Bull. Chem. Soc. Jpn.* **1996**, 207–215.
35. Skarzewski, J.; Gupta, A. *Tetrahedron: Asymmetry* **1997**, *8*, 1861–1867.
36. Prasad, A. S. B.; Kanth, J. V. B.; Periassamy, M. *Tetrahedron* **1992**, *48*, 4623–4628.