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ORIGINAL PAPER

Analogues of nucleosides: synthesis of chiral pyrrolidin-2-ones or pyrrolidines-bearing nucleobases

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Abstract Novel analogues of nucleosides tethered on a chiral pyrrolidin-2-one were prepared, but the imide functionality was unstable under basic conditions. Thus, the carbonyl group was removed from pyrrolidin-2-one, and nucleoside analogues bearing a pyrrolidine ring were synthesized, which were unaffected under basic conditions. A nucleoside dimer was also obtained, bearing a carbamate linkage between two units.

Keywords Imides · Pyrrolidin-2-ones · Pyrrolidines · Carbamate linkage · Nucleosides

Introduction

The use of nucleic acids in therapeutics [1, 2] and bio- and nanotechnologies [3] is troubled by denaturation and/or biodegradation [4], whereas introduction of non-natural nucleosides may afford improved in vivo half-life [5], better structural stability [6–11], or novel interacting groups [12–15]. Thus, a large number of these latter compounds have been prepared to seek out new antiviral and anticancer agents. Among them, 2′,3′-dideoxynucleosides (ddNs) were the most effective therapeutic agents against human immunodeficiency virus (HIV) and hepatitis B virus (HBV) [16, 17], together with 3′-azido-2′,3′-dideoxythymidine (AZT) [18], 2′,3′-dideoxyinosine (DDI) [19], and 2′,3′-dideoxycytidine (DDC) [20]. Moreover, since the glycosidic linkage of these compounds can undergo both acidic and enzymatic hydrolysis, carbocyclic derivatives were further introduced, in which the replacement of the oxygen atom by a methylene group resulted in higher metabolic and chemical stability, often leading to lower cytotoxicity and increased bioavailability [21–23].

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Results and discussion

In recent years, starting from functionalized *trans*-3,4-disubstituted pyrrolidin-2-ones **1a**, **1b** (Fig. 1), we prepared a lot of conformationally restricted amino acids analogues [24, 25] and monomers of foldamers [26]. Moreover, the 2-oxo functionality was easily reduced leading to the corresponding pyrrolidines, whose five-membered ring is isosteric with the carbohydrate unit of nucleosides. This gave rise to interesting biological properties, like improved resistance against enzymes and increased hydrolytic stability [27, 28].

Thus, we devised that pyrrolidin-2-ones **1a**, **1b** [24] could be useful starting material in order to prepare monomeric structures bearing nucleobases and/or to build nanostructured systems. In fact, according to our previous work, we planned to insert these monomers into foldamers already synthesized in our laboratory, directed to obtaining novel structures able to recognize nucleic acid portions or to generate strong interchain interactions.

At first, the chiral inducer at nitrogen atom was removed from compound **1a** to give **2**, and subsequent acylation at N-1 with chloroacetyl chloride afforded the imide **3** in moderate yield. This latter, by reaction with thymine or adenine in presence of NaI and K_2CO_3, afforded in low yield derivatives **4** and **5**, respectively (Scheme 1). Although we planned to insert these products into already prepared foldameric structures, we were disappointed by their low stability. In fact, cleavage of the imido group

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largely occurred even under mild conditions required for ester hydrolysis, thus making problematic the use of these compounds for the synthesis of either diagnostic or nanostructured foldamers stable in biological environment.

Thus, we turned our attention toward pyrrolidin-2-one **1b,** and we devised it could be easily converted in few steps into diprotected nucleoside analogues **6**, tethered on a pyrrolidine ring (Fig. 2). In fact, compound **1b** was fully reduced with $LiAlH_4$ in refluxing THF, to give in good yield the corresponding amino alcohol **7** that, by reaction with Boc_2O, was in turn converted into its Boc derivative **8**. Subsequent benzoylation afforded derivative **9** and eventual removal of the chiral phenylethyl group, by reaction with 1-chloroethylchlorocarbonate, led in good yield to diprotected 3,4-*trans*-disubstituted pyrrolidine **10**, used in the next step without any further purification (Scheme 2).

Then, with the aim of synthesizing monomers **6**, we prepared acetic acid derivatives **11**–**14** bearing a nucleobase (thymine, cytosine, adenine, and guanine) according to literature methods. Thus, (thymin-1-yl)acetic acid **11** and the Cbz-cytosine derivative **12** were prepared starting from thymine and Cbz-substituted cytosine [29], respectively. Adenylacetic acid **13** was prepared starting from adenine [30], and (2-amino-6-benzyloxypurin-9-yl)acetic acid **14**, precursor of guanylacetic acid, arose from 2-amino-6-benzyloxypurine [31] (Fig. 3).

The preparation of monomers is outlined in Scheme 3. The reaction of pyrrolidine derivative **10** with the substituted acetic acids **11**–**14**, carried out in presence of EDC, gave in moderate yield the four orthogonally protected analogues of natural nucleosides **6a**–**6d**. Having in hands compounds **6a**–**6d**, we directed our efforts toward the synthesis of dimer **15**, in which a carbamate linkage was chosen in place of a phosphate group in order to obtain a stable structure between two **6a** units. At first, removal of Boc-protecting group from compound **6a**, on treatment with trifluoroacetic acid (TFA) in DCM, gave the corresponding ammonium salt **15** in good yield. On the other hand, cleavage of the benzoyl group of **6a**, carried out with polymer-supported hydroxide anion in methanol [32], led to the hydroxymethyl derivative **16** that in turn reacted with phosgene in dry THF leading to the corresponding chlorocarbonate **17** (Scheme 4). Eventually, dimer **18**, bearing a carbamate bridge highly resistant against hydrolytic cleavage, was obtained in moderate yield by reaction of compounds **15** and **17** in dichloromethane in presence of TEA at room temperature (Scheme 5).

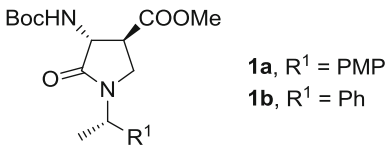
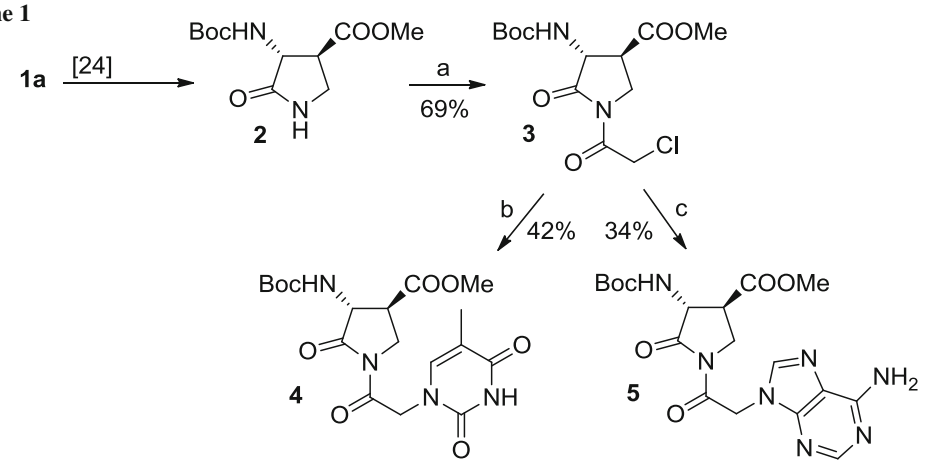


Fig. 1 *trans*-3,4-Disubstituted pyrrolidin-2-ones used as starting materials

Conclusion

In summary, with the aim of obtaining novel compounds able to give self-assembly into well-ordered nanoscale systems, useful for therapy and diagnostics, we prepared novel nucleoside analogues **6a**–**6d**, tethered on a pyrrolidine ring isosteric of deoxyribose. In addition, the orthogonally protected dinucleoside analogue **18**, displaying two units linked through a carbamate bridge—a

Scheme 1



(a) n-BuLi, chloroacetyl chloride, dry THF, -78 °C; (b) thymine, K_2CO_3, NaI, dry DMF, rt; (c) adenine, K_2CO_3, NaI, dry DMF, rt

functional group inert in a biological environment—was prepared in moderate overall yield. The incorporation of these analogues into foldamers, directed toward buildup of nanostructured systems, is currently under investigation, and results will be reported in due course.

Experimental

^{1}H and ^{13}C NMR spectra were determined on a Varian Gemini 200 spectrometer at 200 and 50 MHz for ^{1}H and ^{13}C, respectively, and on a Varian MR 400 spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ unless otherwise reported. Chemical shifts are given as ppm from tetramethylsilane, and *J* values are given in Hertz. Optical rotations, $[\alpha]_D$, were recorded at room temperature on a Perkin-Elmer Model 241 polarimeter at the sodium D line (concentration in g/100 cm^3). LC electrospray ionization mass spectra were obtained with a Finnigan Navigator LC/MS single-quadrupole mass spectrometer, cone voltage 25 V and capillary voltage 3.5 kV, injecting samples dissolved in methanol. Elemental analyses were performed with a Carlo Erba CHN Elemental Analyzer, and their results were found to be in good agreement (±0.3 %) with the calculated values. Column chromatography was performed using Kieselgel 60 Merck (230–400 mesh ASTM). Tetrahydrofuran, dichloromethane, methanol, and DMF were distilled from sodium-benzophenone, calcium hydride, sodium, and phosphorus pentoxide, respectively, under an argon atmosphere. Compounds **1a**, **1b**, and **2** were obtained according to Ref. [24]. Compounds **11–14** were obtained according to Ref. [29–31]. All ^{1}H NMR and ^{13}C NMR spectra were found to be identical with the ones described in the cited references.

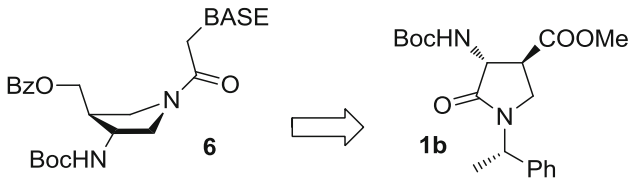


Fig. 2 Nucleosides analogues prepared from pyrrolidin-2-one **1b**

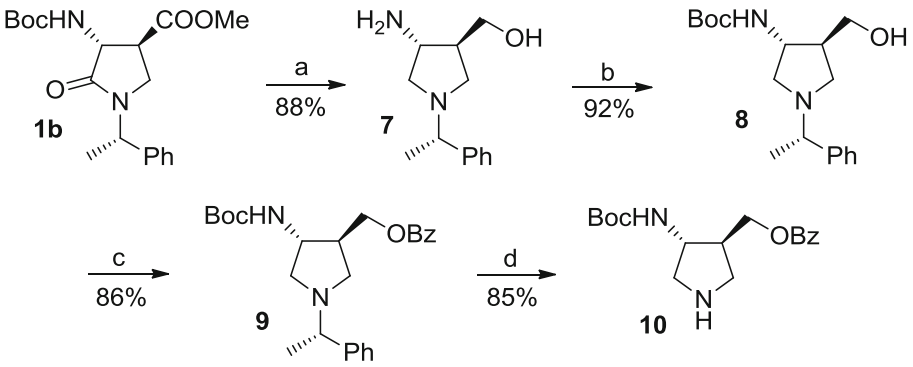
(3S,4R)-Methyl 4-(t-butoxycarbonylamino)-1-(2-chloroacetyl)-5-oxopyrrolidine-3-carboxylate (**3**, $C_{13}H_{19}ClN_2O_6$)

To 776 mg of compound **2** (3 mmol) [24] dissolved in 12 cm^3 dry THF at −78 °C under argon atmosphere, 2.07 cm^3 n-BuLi (1.6 M solution in hexane) was added. After 30 min, 0.33 cm^3 chloroacetyl chloride (4.2 mmol) dissolved in 6 cm^3 dry THF was slowly dropped, and the reaction was stirred for 1 h. Then, the mixture was poured in water and extracted with ethyl acetate (3 × 75 cm^3) and the organic layer was washed with brine and dried (Na_2SO_4). Volatiles were removed, and the residue was purified by silica gel chromatography (cyclohexane:ethyl acetate 70:30) to give 692 mg (69 %) **3**. Colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.43$ (s, 9H), 3.53 (m, 1H), 3.67 (dd, 1H, $J = 9.6$, 11.5 Hz), 3.78 (s, 3H), 4.22 (dd, 1H, $J = 9.6$, 11.5 Hz), 4.35 (dd, 1H, $J = 7.2$, 10.2 Hz), 4.70 (s, 2H), 5.31 (d, 1H, $J = 7.2$ Hz, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 28.6$, 41.1, 43.4, 52.6, 52.9, 56.3, 81.3, 155.8, 166.4, 167.9, 171.1 ppm; ESI–MS: $m/z = 357.1$ ($[M + Na]^+$); $[\alpha]_D^{20} = -21.6°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

*General procedure for preparation of compounds **4** and **5***

To a solution of 335 mg compound **3** (1 mmol) in 3 cm^3 dry DMF, 252 mg thymine (2 mmol) or 270 mg adenine (2 mmol), 691 mg dry K_2CO_3 (5 mmol), and 75 mg NaI

Scheme 2



(a) $LiAlH_4$, refluxing dry THF; (b) Boc_2O, TEA, MeOH, rt; (c) PhCOCl, DMAP, dry DCM, rt; (d) $CH_3CHClCOCl$, dry DCM, 0 °C, then refluxing dry MeOH

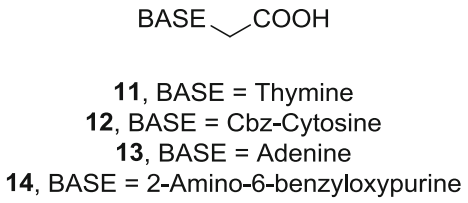
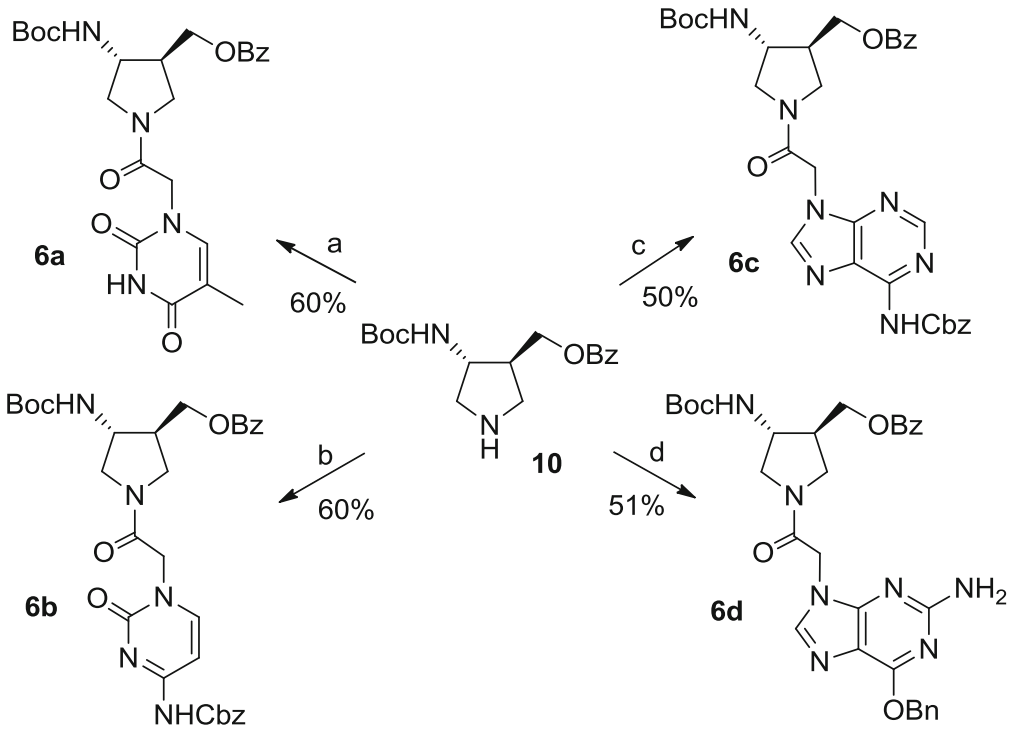


Fig. 3 Nucleobase-substituted acetic acids **11–14**

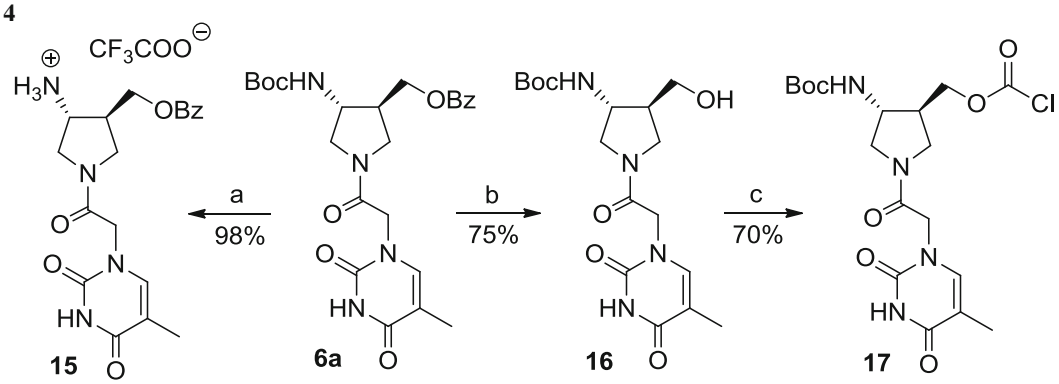
(0.5 mmol) were added at once. After 4 h at rt, the mixture was poured in water and extracted with ethyl acetate (3×25 cm^3), and combined organic layers were washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (cyclohexane : ethyl acetate 80:20) to give compound **4** or **5**.

Scheme 3



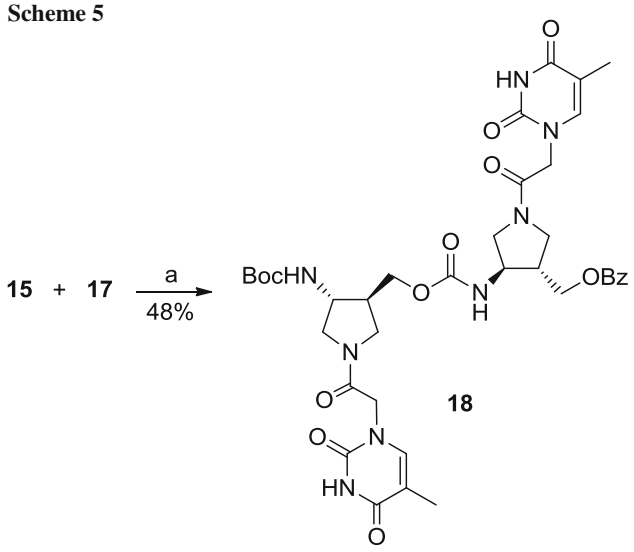
(a) Acid **11**, TEA, EDC, dry DCM:dry DMF 4:1, rt; (b) acid **12**, TEA, EDC, dry DCM:dry DMF 4:1, rt; (c) acid **13**, TEA, EDC, dry DCM:dry DMF 4:1, rt; (d) acid **14**, TEA, EDC, dry DCM:dry DMF 4:1, rt

Scheme 4



(a) TFA, dry DCM, rt; (b) IRA 900 in the hydroxide form, MeOH, rt; (c) $COCl_2$, dry THF, rt

Scheme 5



(3S,4R)-Methyl 4-(t-butoxycarbonylamino)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]-5-oxopyrrolidine-3-carboxylate (**4**, $C_{18}H_{24}N_4O_8$)

According to the above-reported general procedure, 178 mg (42 %) **4** was obtained. Colorless viscous oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.45$ (s, 9H), 1.92 (s, 3H), 3.55 (m, 1H), 3.68 (m, 1H), 3.79 (s, 3H), 4.17 (dd, 1H, $J = 9.2$, 11.7 Hz), 4.41 (dd, 1H, $J = 7.3$, 9.9 Hz), 4.95 (ABq, 2H, $J = 7.4$ Hz), 5.39 (d, 1H, $J = 6.9$ Hz, NH), 6.88 (s, 1H), 8.58 (br s, 1H, NH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 28.2$, 41.9, 43.2, 52.4, 52.7, 56.4, 81.0, 140.5, 151.0, 155.1, 164.3, 167.4, 171.3, 171.7 ppm; ESI–MS: $m/z = 447.2$ ($[M + Na]^+$); $[\alpha]_D^{20} = -16.4°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

(3S,4R)-Methyl 1-[2-(6-amino-9H-purin-9-yl)acetyl]-4-(t-butoxycarbonylamino)-5-oxopyrrolidine-3-carboxylate (**5**, $C_{18}H_{23}N_7O_6$)

According to the above-reported general procedure, 148 mg (34 %) **5** was obtained. Colorless viscous oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.44$ (s, 9H), 3.56 (m, 1H), 3.65 (m, 1H), 3.76 (s, 3H), 4.17 (m, 1H), 4.42 (m, 1H), 5.56 (m, 2H), 5.85 (br s, 1H, NH), 6.07 (br s, 2H, NH), 7.80 (s, 1H), 8.30 (s, 1H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 28.4$, 29.8, 41.8, 43.4, 48.0, 52.9, 56.6, 81.3, 119.1, 141.3, 150.4, 153.2, 155.8, 167.1, 171.6 ppm; ESI–MS: $m/z = 434.2$ ($[M + H]^+$), 456.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -12.3°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-4-Amino-1-[(S)-1-phenylethyl]pyrrolidin-3-yl]methanol (**7**, $C_{13}H_{20}N_2O$)

To a solution containing 2.90 g of compound **1b** [24] (8.0 mmol) in 20 cm^3 dry THF at rt, 16 cm^3 of 2 M solution of $LiAlH_4$ in dry THF (32 mmol) was added under inert atmosphere, and the mixture was refluxed for 3 h. Then, 10 cm^3 methanol and 60 cm^3 aqueous saturated NH_4Cl solution were sequentially added, and the mixture was extracted with ethyl acetate (3 × 200 cm^3). The organic layer was dried (Na_2SO_4), filtered, and then concentrated under reduced pressure. Purification of the residue by silica gel chromatography (ethyl acetate:methanol 8:2) gave 1.55 g (88 %) **7**. Colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.38$ (d, $J = 7.2$ Hz, 3H), 1.90–2.04 (m, 1H), 2.21 (dd, $J = 4.2$, 5.8 Hz, 1H), 2.41–2.59 (m, 4H, 1H + OH + NH_2), 2.64–2.85 (m, 2H), 3.33 (q, $J = 7.2$ Hz, 1H), 3.28–3.38 (m, 1H), 3.69 (d, $J = 6.8$ Hz, 2H), 7.24–7.35 (m, 5ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 16.3$, 41.0, 41.6, 49.8, 60.7, 61.9, 126.8, 127.9, 128.7, 139.3, 170.4 ppm; ESI–MS: $m/z = 221.2$ ($[M + H]^+$), 243.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -32.3°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

(3R,4S,1′S)-3-(t-Butoxycarbonylamino)-4-(hydroxymethyl)-1-(1-phenylethyl)pyrrolidine (**8**, $C_{18}H_{28}N_2O_3$)

To 1.48 g of compound **7** (6.7 mmol) and 1.1 cm^3 TEA (8 mmol) in 14 cm^3 methanol, 1.6 g Boc_2O (7.4 mmol) was added, and the reaction was stirred for 2 h at room temperature. After removal of methanol under reduced pressure, 10 cm^3 water and 100 cm^3 ethyl acetate were added, the organic layer was separated, and the aqueous phase was extracted with ethyl acetate (2 × 100 cm^3). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated to give 1.98 g (92 %) of compound **8**. Colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 3.49$ (d, $J = 6.6$ Hz, 3H), 1.42 (s, 9H), 2.03–2.19 (m, 2H), 2.55–2.77 (m, 3H), 3.17 (q, $J = 6.6$ Hz, 1H), 3.54 (d, $J = 5.9$ Hz, 2H), 3.56 (br s, 1H, OH), 3.79–3.92 (m, 1H), 5.13 (d, $J = 7.3$ Hz, 1H, NH), 7.18–7.34 (m, 5ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 22.5$, 28.4, 53.9, 55.2, 58.5, 64.5, 65.1, 80.0, 127.0, 127.2, 128.4, 144.3, 156.1 ppm; ESI–MS: $m/z = 321.2$ ($[M + H]^+$), 343.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -37.6°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

t-Butyl [(3R,4S)-4-(hydroxymethyl)-1-[(S)-1-phenyl ethyl]pyrrolidin-3-yl]carbamate (**9**, $C_{25}H_{32}N_2O_4$)

To a solution containing 1.98 g of compound **8** (6.2 mmol) in 13 cm^3 dry dichloromethane, 72 mg DMAP (0.6 mmol) was added at room temperature, followed by 0.79 cm^3 benzoyl chloride (6.8 mmol). After stirring for 1 h, 20 cm^3 water and 50 cm^3 ethyl acetate were added, the organic layer was separated, and the aqueous phase was extracted with ethyl acetate (2 × 50 cm^3). The combined organic layers were washed with 10 cm^3 of a saturated $NaHCO_3$ solution, dried (Na_2SO_4), filtered, concentrated, and the residue was purified by silica gel chromatography (cyclohexane:ethyl acetate 9:1) to give 2.26 g (86 %) of

compound **9**. Low-melting white solid; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.35$ (d, $J = 6.6$ Hz, 3H), 1.43 (s, 9H), 2.10–2.27 (m, 1H), 2.29–2.44 (m, 1H), 2.67–2.84 (m, 3H), 3.22 (q, $J = 6.6$ Hz, 1H), 4.01–4.16 (m, 1H), 4.34 (dd, $J = 1.9$, 6.3 Hz, 2H), 5.00 (d, $J = 7.3$ Hz, 1H, NH), 7.18–7.33 (m, 4ArH), 7.35–7.59 (m, 4ArH), 7.99–8.06 (m, 2ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 22.6$, 28.3, 45.3, 52.7, 54.8, 64.8, 65.8, 79.2, 126.9, 128.2, 128.3, 128.4, 129.5, 132.8, 144.4, 155.2, 166.3 ppm; ESI–MS: *m/z* = 425.2 ($[M + H]^+$), 447.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -19.2°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-4-(t-Butoxycarbonylamino)pyrrolidin-3-yl]methyl benzoate (**10**, $C_{17}H_{24}N_2O_4$)

To a solution containing 2.26 g of compound **9** (5.3 mmol) in 16 cm^3 dry dichloromethane at 0 °C, 1.17 cm^3 1-chloroethyl chloroformate (10.8 mmol) was added, and after 30 min, the solvent was removed under reduced pressure. The residue was dissolved in 10 cm^3 methanol, and the solution was refluxed for 40 min. After removal of the volatiles, the residue was dissolved in 15 cm^3 AcOEt, and the organic layer washed with 10 cm^3 of a saturated solution of Na_2CO_3. The aqueous phase was extracted with additional 50 cm^3 AcOEt and, after separation, the organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. Eventually, the residue was purified by silica gel chromatography (ethyl acetate:methanol 8:2), to give 1.45 g (85 %) of compound **10**. Colorless oil; ^{1}H NMR (200 MHz, $CDCl_3$): $\delta = 1.40$ (s, 9H), 2.35–2.49 (m, 1H), 2.75–2.94 (m, 2H), 3.19–3.41 (m, 2H), 3.94–4.09 (m, 1H), 4.31 (dd, $J = 7.0$, 11.2 Hz, 1H), 4.41 (dd, $J = 6.0$, 11.2 Hz, 1H), 4.81 (br s, 1H, NH), 5.39 (d, $J = 6.2$ Hz, 1H, NH), 7.32–7.45 (m, 2ArH), 7.47–7.58 (m, 1ArH), 7.95–8.04 (m, 2ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 28.3$, 45.8, 48.7, 52.9, 54.3, 65.2, 79.6, 128.3, 129.5, 133.0, 155.4, 166.3 ppm; ESI–MS: *m/z* = 321.2 ($[M + H]^+$), 343.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -42.6°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

General procedure for preparation of compounds ***6a–6d***

To a solution containing 640 mg of compound **10** (2 mmol) in 4 cm^3 dry dichloromethane and 1 cm^3 dry DMF, acids **11**-**14** (2.2 mmol) were added, followed by 0.14 cm^3 TEA (1 mmol) and 460 mg EDC (2.4 mmol). After stirring for 12 h at room temperature, 8 cm^3 0.1 M HCl and 20 cm^3 ethyl acetate were added, the organic layer was separated and the extraction with ethyl acetate was repeated twice (2 × 40 cm^3). The combined organic layers were washed with a 10 cm^3 of a saturated solution of $NaHCO_3$ and dried (Na_2SO_4). After removal of the solvents under reduced pressure, the residue was chromatographed on silica gel (ethyl acetate:methanol 9:1) to give the products **6a–6d**.

[(3S,4R)-4-(t-Butoxycarbonylamino)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-yl]methyl benzoate (**6a**, $C_{24}H_{30}N_4O_7$)

According to the above-reported general procedure, 730 mg (75 %) of compound **6a** was obtained by reaction of **10** with acid **11** [29]. White solid; m.p.: 138–139 °C; ^{1}H NMR (200 MHz, $CDCl_3$, mixture of rotamers): $\delta = 1.42$ (s, 9H), 1.88 (s, 3H), 2.45–2.62 (m, 1H, 55 %), 2.63–2.78 (m, 1H, 45 %), 3.21–3.53 (m, 2H), 3.75–4.51 (m, 7H), 5.65 (d, $J = 7.1$ Hz, 1H), 7.00 (s, 1Het-H), 7.35–7.47 (m, 2ArH), 7.48–7.56 (m, 1ArH), 7.93–8.04 (m, 2ArH), 9.80 (s, 1H, 45 %), 9.83 (s, 1H, 55 %) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, mixture of rotamers): $\delta = 12.2$, 28.3, 41.9, 44.5, 47.4, 48.4, 48.6, 50.8, 51.1, 52.8, 63.7, 63.8, 80.2, 110.7, 128.4, 129.6, 130.1, 133.3, 140.9, 151.2, 155.3, 155.4, 164.1, 165.0, 166.2, 166.3 ppm; ESI–MS: *m/z* = 509.2 ($[M + Na]^+$); $[\alpha]_D^{20} = -38.7°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-1-[2-[4-(Benzyloxycarbonylamino)-2-oxo-pyrimidin-1(2H)-yl]acetyl]-4-(t-butoxycarbonylamino)-pyrrolidin-3-yl]methyl benzoate
(**6b**, $C_{31}H_{35}N_5O_8$)

According to the above-reported general procedure, 764 mg of compound **6b** (63 %) was obtained by reaction of **10** with acid **12** [29]. White solid; m.p.: 67–68 °C; ^{1}H NMR (200 MHz, $CDCl_3$, mixture of rotamers): $\delta = 1.42$ (s, 9H), 2.45–2.71 (m, 1H), 3.22–4.39 (m, 8H), 4.47 (dd, $J = 5.0$, 11.0 Hz, 1H), 4.88 (br s, 1H, NH), 5.19 (s, 2H), 5.45 (br s, 1H, NH), 7.19–7.64 (m, 8ArH + 2Het-H), 7.95–8.05 (m, 2ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, mixture of rotamers): $\delta = 28.4$, 38.3, 44.5, 44.7, 46.8, 47.6, 50.9, 51.3, 52.2, 52.3, 63.8, 64.0, 67.9, 68.1, 80.4, 80.7, 95.3, 127.2, 128.1, 128.3, 128.4, 128.5, 128.6, 128.8, 129.6, 130.1, 130.8, 133.5, 135.2, 149.6, 152.8, 153.1, 155.7, 155.9, 163.5, 164.9, 165.2, 166.2, 169.2 ppm; ESI–MS: *m/z* = 628.3 ($[M + Na]^+$); $[\alpha]_D^{20} = -56.7°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-1-[2-[6-(Benzyloxycarbonylamino)-9H-purin-9-yl]acetyl]-4-(t-butoxycarbonylamino)pyrrolidin-3-yl]-methyl benzoate (**6c**, $C_{32}H_{35}N_7O_7$)

According to the above-reported general procedure, 630 mg (50 %) of compound **6c** was obtained by reaction of **10** with acid **13** [30]. White solid; m.p.: 81–82 °C; ^{1}H NMR (200 MHz, $CDCl_3$, mixture of rotamers): $\delta = 1.42$ (s, 9H, 45 %), 1.44 (s, 9H, 55 %) 2.51–2.66 (m, 1H, 55 %), 2.69 (m, 1H, 45 %), 3.27–3.47 (m, 1H), 3.48–3.65 (m, 1H), 3.79 (s, 2H, 55 %), 3.82–4.03 (m, 1H), 4.05–4.25 (m, 1H), 4.26–4.37 (m, 1H), 4.42 (dd, $J = 5.3$, 11.2 Hz, 1H), 4.53

(dd, $J = 4.4$, 11.2 Hz, 1H), 4.92 (br s, 1H, NH), 4.96 (s, 2H, 45 %), 5.27 (s, 2H, 55 %), 5.29 (s, 2H, 45 %), 7.28–7.64 (m, 8ArH + 1Het-H), 7.96–8.06 (m, 2ArH), 8.15 (br s, 1H, NH), 8.69 (s, 1Het-H, 55 %), 8.72 (s, 1Het-H, 45 %) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, mixture of rotamers): $\delta = 28.3$, 44.1, 44.5, 44.6, 44.7, 47.5, 51.0, 51.1, 53.0, 51.1, 53.0, 63.6, 63.7, 67.8, 80.5, 121.0, 128.5, 128.6, 129.5, 129.6, 133.3, 133.4, 135.5, 143.2, 144.2, 149.3, 151.0, 151.5, 152.8, 153.0, 155.1, 155.2, 164.0, 166.2 ppm; ESI–MS: $m/z = 652.3$ ($[M + Na]^+$); $[\alpha]_D^{20} = -26.3°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-1-[2-(2-Amino-6-benzyloxy-9H-purin-9-yl)-acetyl]-4-(t-butoxycarbonylamino)pyrrolidin-3-yl]-methyl benzoate (**6d**, $C_{31}H_{35}N_7O_6$)

According to the above-reported general procedure, 614 mg (51 %) of compound **6d** was obtained by reaction of **10** with acid **14** [31]. Yellow solid; m.p.: 57–58 °C; ^{1}H NMR (200 MHz, $CDCl_3$, mixture of rotamers): $\delta = 1.39$ (s, 9H, 35 %), 1.41 (s, 9H, 65 %), 2.41–2.68 (m, 1H), 3.22–3.49 (m, 2H), 3.73–3.89 (m, 2H), 4.02–4.18 (m, 1H), 4.24 (dd, $J = 6.2$, 11.5 Hz, 1H), 4.41 (dd, $J = 4.9$, 11.5 Hz, 1H), 4.67 (s, 2H, 65 %), 4.73 (s, 2H, 35 %), 5.03 (br s, NH_2, 65 %), 5.14 (br s, 1H, NH), 5.44 (br s, 2H, NH_2, 35 %), 5.59 (s, 2H, 35 %), 5.51 (s, 2H, 65 %), 7.21–7.62 (m, 8ArH), 7.67 (s, 1Het-H), 7.94–8.05 (m, 2ArH) ppm; ^{13}C NMR (50 MHz, $CDCl_3$, mixture of rotamers): $\delta = 28.3$, 44.1, 44.4, 50.7, 50.9, 52.7, 63.7, 68.0, 80.1, 114.7, 127.1, 127.9, 128.1, 128.2, 128.3, 128.4, 129.5, 130.1, 133.2, 133.3, 136.4, 140.2, 140.3, 154.1, 155.2, 155.3, 159.2, 160.9, 164.6, 164.7, 166.2, 166.3 ppm; ESI–MS: $m/z = 624.3$ ($[M + Na]^+$); $[\alpha]_D^{20} = -43.6°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

(3R,4S)-4-[(Benzoyloxy)methyl]-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-aminium 2,2,2-trifluoroacetate (**15**, $C_{21}H_{23}F_3N_4O_7$)

To a solution containing 190 mg of compound **6a** (0.4 mmol) in 3 cm^3 dry dichloromethane, 1.5 cm^3 TFA was slowly added and the clear solution was stirred for 1 h at rt. After removal of organics under reduced pressure, the residue was co-evaporated with ethyl ether (3 × 5 cm^3) to give the compound 196 mg (98 %) of compound **15**. Colorless oil, used in the next step without any further purification. ^{1}H NMR (400 MHz, CD_3OD, mixture of rotamers): $\delta = 1.87$ (s, 3H), 2.80–2.93 (m, 1H, 40 %), 2.93–3.05 (m, 1H, 60 %), 3.53–3.81 (m, 2H), 3.86–4.18 (m, 4H), 4.40–4.68 (m, 3H), 7.30 (s, 1Het-H), 7.48–7.56 (m, 2ArH), 7.61–7.68 (m, 1ArH), 8.03–8.10 (m, 2ArH) ppm; ESI–MS: $m/z = 387.3$ ($[M\text{-}CF_3COO]^+$), 409.2 ($[M\text{-}CF_3COOH + Na]^+$); $[\alpha]_D^{20} = +54.2°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

t-Butyl [(3R,4S)-4-(hydroxymethyl)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-yl]carbamate (**16**, $C_{17}H_{26}N_4O_6$)

A solution of 486 mg of compound **6a** (1 mmol) in 2 cm^3 methanol was added at rt under argon atmosphere to a suspension of 0.5 g Amberlite IRA 900 in the hydroxide form [32] in 2 cm^3 methanol. After stirring for 6 h, the resin was filtered off and washed with 15 cm^3 methanol. The solvent was evaporated under reduced pressure, and the residue was washed with 10 cm^3 ethyl ether to afford 270 mg (70 %) of compound **16**. White solid; m.p.: 67–68 °C; ^{1}H NMR (200 MHz,$CDCl_3$, mixture of rotamers): $\delta = 1.46$ (s, 9H, 55 %), 1.49 (s, 9H, 45 %), 1.86 (s, 3H, 55 %), 1.88 (s, 3H, 45 %), 2.18–2.31 (m, 1H, 55 %), 2.33–2.44 (m, 1H, 45 %), 3.18–3.41 (m, 1H), 3.46–4.12 (m, 6H), 4.52 (s, 2H, 55 %), 4.56 (s, 2H, 45 %), 7.31 (s, 1Het-H) ppm; ESI–MS: $m/z = 405.2$ ($[M + Na]^+$); $[\alpha]_D^{20} = +27.4°$ cm^2 g^{-1} ($c = 0.5$, $CHCl_3$).

[(3S,4R)-4-(t-Butoxycarbonylamino)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-yl]methyl carbonochloridate (**17**, $C_{18}H_{25}ClN_4O_7$)

To a solution containing 246 mg **16** (0.64 mmol) in 3 cm^3 dry THF under argon atmosphere at rt, 0.67 cm^3 of 20 % phosgene solution in toluene (1.28 mmol) was added. After stirring for 5 h, organics were evaporated at low temperature under reduced pressure, to give directly 201 mg **17** (70 %). Low-melting white solid; ^{1}H NMR (400 MHz, $CDCl_3$, mixture of rotamers): $\delta = 1.46$ (s, 9H), 1.93 (s, 3H), 2.45–2.60 (m, 1H, 50 %), 2.60–2.75 (m, 1H, 50 %), 3.30 (dd, $J = 8.4$ Hz, 4.0 Hz, 1H), 3.43–3.54 (m, 1H), 3.87–4.02 (m, 2H), 4.02–4.40 (m, 3H), 4.40–4.61 (m, 2H), 4.69–4.83 (m, NH, 50 %), 4.87–4.99 (m, NH, 50 %), 7.15 (s, 1Het-H, 50 %), 7.29 (s, 1Het-H, 50 %), 8.47 (s, 1H, NH, 50 %), 8.52 (s, 1H, NH, 50 %) ppm; ESI–MS: $m/z = 467.9$ ($[M + Na]^+$); $[\alpha]_D^{20} = -21.5°$ cm^2 g^{-1} (c = 0.5, $CHCl_3$).

[(3S,4R)-4-[[[[(3S,4R)-4-(t-Butoxycarbonylamino)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-yl)methoxy)carbonyl)amino)-1-[2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)acetyl]pyrrolidin-3-yl]methyl benzoate (**18**, $C_{37}H_{46}N_8O_{12}$)

To a solution containing 196 mg of compound **15** (0.39 mmol) in 1.5 cm^3 dry dichloromethane, 0.11 cm^3 TEA (0.8 mmol) and then 179 mg of compound **17** (0.4 mmol) were added, and the mixture was stirred for 12 h at room temperature. Then, 1 cm^3 of 1 M HCl was added, and the mixture was extracted with ethyl acetate (3 × 10 cm^3). After drying (Na_2SO_4) and removal of the solvents, the residue was purified by precipitation from dichloromethane to give 149 mg (48 %) of compound **18**. Low-melting spongy solid; ^{1}H NMR (400 MHz, CD_3OD,

mixture of rotamers): $\delta = 1.44$ (s, 9H, 40 %), 1.45 (s, 9H, 60 %), 1.88 (s, 6H), 2.20–2.30 (m, 1H, 40 %), 2.32–2.44 (m, 1H, 60 %), 2.61–2.72 (m, 1H, 60 %), 2.72–2.83 (m, 1H, 40 %), 3.28–3.36 (m, 2H), 3.44–3.85 (m, 6H), 3.88–4.13 (m, 2H + 2H, 40 %), 4.37 (dd, $J = 7.0$, 7.8 Hz, 2H, 60 %), 4.39–4.69 (m, 6H), 7.30–7.32 (m, 2Het-H), 7.48–7.52 (m, 2ArH), 7.59–7.67 (m, 1ArH), 8.01–8.08 (m, 2ArH) ppm; ^{13}C NMR (100 MHz, CD_3OD, mixture of rotamers): $\delta = 12.2$, 12.3, 12.4, 28.3, 28.4, 41.2, 41.4, 42.0, 44.4, 44.5, 44.7, 48.3, 48.4, 48.6, 48.8, 50.8, 51.0, 51.2, 52.5, 52.7, 52.9, 61.2, 61.4, 63.7, 63.8, 80.3, 110.7, 110.8, 111.0, 128.4, 128.5, 129.6, 129.7, 130.2, 133.2, 140.8, 140.9, 151.0, 151.2, 151.3, 155.3, 155.4, 155.8, 156.0, 164.0, 164.1, 164.9, 166.1, 166.2, 166.4 ppm; ESI–MS: $m/z = 817.3$ ($[M + Na]^+$); $[\alpha]_D^{20} = -32.6°$ cm^2 g^{-1} ($c = 0.3$, $CHCl_3$).

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