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Novel synthetic routes to *N*-(2-amino-9*H*-purin-6-yl)-substituted amino acids

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Experimental

2-Amino-6-chloro-9*H*-purine (**1**), *N*,*N*-dimethylacetamide (DMA) and *tert*-butyl (*S*)-phenylalaninate hydrochloride are commercially available. 2-Acetylamino-6-chloro-9*H*-purine (**2**),[S1] 6-chloro-2-formylamino-9*H*-purine (**3**),[S2] 2-*tert*-butoxycarbonylamino-6-chloro-9*H*-purine (**4**),[S3] *tert*-butyl (*R*)- and (*S*)-valinate acetates[S4] were synthesized according to the described procedures. 6-Chloro-2-trifluoroacetylamino-9*H*-purine (**5**) was synthesized for the first time similarly to 6-chloro-2-trifluoroacetylamino-9-isopropyl-9*H*-purine.[S5]

Flash column chromatography was performed using Silica gel 60 (0.063–0.040 mm, Alfa Aesar). Melting points were obtained on a SMP3 apparatus (Barloworld Scientific, UK) and are uncorrected. Optical rotations were measured on a Perkin Elmer M341 polarimeter. The ^{1}H and ^{19}F NMR spectra were recorded on a Bruker DRX-400 instrument (400 MHz and 376 MHz, respectively) in DMSO-d_6 with TMS and hexafluorobenzene as internal references at room temperature or at 100 °C (compound **7**). The ^{13}C NMR spectra were recorded on a Bruker Avance 500 instrument (125 MHz) in DMSO-d_6 with TMS as an internal reference. Mass spectra were recorded with preliminary chromatographic separation on a quadrupole Shimadzu LCMS-2010 system; working needle voltage 4.5 kV; chemical ionization under atmospheric pressure (APCI, 400 °C) or electrospray ionization (ESI); nitrogen as a carrier gas; flow rate 2.5 L/min. The other parameters of LCMS experiment (columns, elution rates, eluents) and the retention times (τ_R) are specified for each compound. The HRMS spectra were obtained on a Bruker Daltonics series MicrOTOF-Q II mass spectrometer, electrospray ionization with direct sample inlet (flow rate 180 μL/h). The mass spectrometer was operated in the positive ionization mode in the mass range of 50-800 Da at a capillary temperature of 250 °C. Reversed-phase HPLC was performed using an Agilent 1100 chromatograph; column Phenomenex Luna C(18), 250×4.6 mm, 5 μm; MeCN-0.2% aq. CF_3CO_2H mixtures as an eluent (gradient from 10:90 to 90:10 for 30 min); flow rate was 0.8 mL/min; detection at 220,

230, and 254 nm. Chiral reversed-phase HPLC analysis of compounds (*R*)-**11** and (*S*)-**11** was performed using an Agilent 1200 chromatograph; ChiraDex column, MeCN–H_2O 8:2 mixture as an eluent; flow rate 0.8 mL/min.

6-Chloro-2-trifluoroacetylamino-9*H*-purine (5). Trifluoroacetic anhydride (0.60 mL, 4.27 mmol) was added to a suspension of compound **1** (0.24 g, 1.42 mmol) in CH_2Cl_2 (5.5 mL) at room temperature. The reaction mixture was stirred for 4 h. The precipitate was filtered off, washed with CH_2Cl_2 and dried *in vacuo* at 100 °C to yield 0.20 g (54%) of compound **5** as a colourless powder. Mp >350 °C. ^{1}H NMR (DMSO-d_6) δ: 8.66 (s, 1H, C^{8}H); 12.39 (s, 1H, NH); 14.01 (br s, 1H, N^{9}H). ^{19}F NMR (DMSO-d_6) δ: 88.47 (s, 3H, CF_3). ^{13}C NMR (DMSO-d_6) δ: 115.39 (q, *J* 289.3 Hz, CF_3), 146.32 (C^5), 148.32 (C^2), 149.63 (C^8), 153.90 (C^4), 154.16 (q, *J* 37.9 Hz, CO), 159.79 (C^6). RP HPLC: τ_R 11.6 min. HRMS for $C_7H_4ClF_3N_5O$ ($M + H^+$), calcd. 266.0056, found 266.0051.

***N*-(2-Acetylamino-9*H*-purin-6-yl)-(*S*)-phenylalanine *tert*-butyl ester hydroacetate (7).** (*S*)-PheO*t*Bu×HCl (1.00 g, 3.88 mmol) and TEA (0.72 mL, 5.17 mmol) were added to a suspension of compound **2** (274 mg, 1.29 mmol) in DMA (3.0 mL). The reaction mixture was heated at 100 °C for 12 h, and then hot water (30 mL) was added. After cooling, the precipitate was separated by centrifugation, washed with water and dried *in vacuo*. The crude product was purified by flash column chromatography on silica gel (eluent from benzene-EtOAc 1:1 to EtOAc). Fractions containing the target product were concentrated to volume of 10 mL and acetic acid (74 μL, 1.29 mmol) was added. The solution was heated to 60 °C, and then hexane (100 mL) was added. The mixture was cooled to 10 °C; the precipitate was filtered off to yield 0.37 g (63%) of compound **7** as colourless crystals. Mp 118–125 °C. $[\alpha]_D^{20}$ –2.85 (*c* 1.0, $CHCl_3$). Anal. calcd. for $C_{22}H_{28}N_6O_5$: C, 57.89; H, 6.18; N, 18.41. Found: C, 58.06; H, 6.12; N, 18.60. ^{1}H NMR (DMSO-d_6, 100 °C) δ: 1.34 (s, 9H, *t*Bu); 1.90 (s, 3H, $C\underline{H}_3CO_2H$); 2.25 (s, 3H, CH_3CO); 3.21 (d, 2H, CH_2-Phe, *J* 6.5 Hz); 5.10 (br s, 1H, CH-Phe); 6.92 (br s, 1H, C$^{6'}$NH); 7.17 (m, 1H, Ph); 7.25 (m, 4H, Ph); 7.90 (s, 1H, C$^{8'}$H); 9.16 (s, 1H, N$\underline{H}$Ac); 11.99 (br s, 2H, N$^{9'}$H, $CH_3CO_2\underline{H}$). ^{13}C NMR (DMSO-d_6) δ: 20.96 ($\underline{C}H_3COOH$); 24.61 ($\underline{C}H_3CONH$); 27.47 (C($\underline{C}H_3)_3$); 36.53 (CH_2-Phe); 54.95 (CH-Phe); 80.61 ($\underline{C}(CH_3)_3$); 115.75 (C$^{5'}$); 126.31 (*p*-Ph); 128.08 (*o*-Ph); 129.11 (*m*-Ph); 137.78 and 138.39 (C$^{8'}$ and *i*-Ph); 150.70 and 152.39 (C$^{4'}$ and C$^{2'}$); 153.81 (C$^{6'}$); 169.40, 171.12 and 171.88 (CO AcNH, AcOH and CO_2*t*-Bu). RP HPLC: τ_R 17.7 min. LC-MS (Phenomenex Luna CN, 150×4.6 mm, 3 μm, eluent MeCN–H_2O 85:15, flow rate 0.8 mL/min): τ_R 5.7 min; *m*/*z* (I_{rel}, %): 341 [M – *t*-Bu +

2H]$^+$ (41.0), 363 [M - *t*-Bu + H + Na]$^+$ (10.4), 397 [M + H]$^+$ (100.0), 419 [M + Na]$^+$ (70.3), 435 [M + K]$^+$ (17.4). Free base $C_{20}H_{24}N_6O_3$ (396.45).

***N*-(2-Amino-9*H*-purin-6-yl)-(*S*)-phenylalanine *tert*-butyl ester (8).** (*S*)-PheO*t*Bu×HCl (0.20 g, 0.78 mmol) and TEA (144 μL, 1.03 mmol) were added to a solution of compound **5** (68.7 mg, 0.26 mmol) in DMA (0.52 mL). The mixture was heated at 100 °C for 12 h, and then hot water (10 mL) was added. After cooling, the precipitate was separated by centrifugation, washed with water and dried *in vacuo*. Yellowish amorphous crude product was purified by flash column chromatography (eluent $CHCl_3$-MeOH from 10:0.1 to 10:0.9) to yield 46 mg (50%) of compound **8** as a yellowish powder. Mp 123 °C. $[\alpha]_D^{25}$ –4.54 (*c* 1.0, $CHCl_3$). ^{1}H NMR (DMSO-d_6) δ: 1.33 (s, 9H, *t*Bu); 3.03-3.23 (m, 2H, CH_2-Phe); 4.77 (br s, 1H, CH-Phe); 5.69 (s, 2H, $C^{2'}NH_2$); 7.03 (br s, 1H, $C^{6'}NH$); 7.20 (m, 1H, Ph); 7.24-7.31 (m, 4H, Ph); 7.68 (s, 1H, $C^{8'}H$); 12.12 (br s, 1H, $N^{9'}H$). ^{13}C NMR (DMSO-d_6) δ: 27.52 (C($\underline{C}H_3)_3$); 36.97 (CH_2-Phe); 54.63 (CH-Phe); 80.46 ($\underline{C}(CH_3)_3$); 112.68 ($C^{5'}$); 126.31 (*p*-Ph); 128.07 (*o*-Ph); 129.16 (*m*-Ph); 135.89 ($C^{8'}$); 137.72 (*i*-Ph); 152.46 and 153.75 ($C^{4'}$ and $C^{2'}$); 159.66 ($C^{6'}$); 171.36 ($\underline{C}O_2t$-Bu). RP HPLC: τ_R 16.6 min. LC-MS (Supelcosil LC18, 250×4.6 mm, 5 μm, eluent MeOH–H_2O 75:25, flow rate 0.8 mL/min): τ_R 6.6 min; *m*/*z* (I_{rel}, %): 253 [M – *t*-Bu – CO_2]$^+$ (5.0), 299 [M – *t*-Bu + 2H]$^+$ (15.8), 355 [M + H]$^+$ (100.0), 387 [M + H + MeOH]$^+$ (1.4). $C_{18}H_{22}N_6O_2$ (354.42). HRMS for $C_{18}H_{23}N_6O_2$ (M + H$^+$), calcd. 355.1882, found 355.1877.

***N*-(2-Amino-9*H*-purin-6-yl)-(*S*)-phenylalanine (9).** A suspension of compound **7** (0.228 g, 0.50 mmol) in 1 N aqueous NaOH (3.0 mL) was stirred at 60 °C for 2 h, then filtered. The filtrate was acidified with 1 N HCl to pH ~ 6. The precipitate was filtered off, washed with water and dried *in vacuo* over P_2O_5 at 100 °C to yield 92 mg (61%) of compound **9** as a colourless solid. Mp 234–236 °C (decomp.). $[\alpha]_D^{30}$ +14.6 (*c* 0.2, DMF). ^{1}H NMR (DMSO-d_6) δ: 3.20 (m, 2H, CH_2-Phe); 4.88 (br s, 1H, CH-Phe); 5.75 (s, 2H, NH_2); 7.00 (br s, 1H, $C^{6'}NH$); 7.17 (m, 1H, Ph); 7.26 (m, 4H, Ph); 7.67 (s, 1H, $C^{8'}H$); 12.2 (br s, 2H, $N^{9'}H$ and CO_2H). ^{13}C NMR (DMSO-d_6) δ: 36.57 (CH_2-Phe); 53.77 (CH-Phe); 112.23 ($C^{5'}$); 126.26 (*p*-Ph); 128.09 (*o*-Ph); 129.07 (*m*-Ph); 135.94 ($C^{8'}$); 138.06 (*i*-Ph); 152.22 ($C^{4'}$); 153.79 ($C^{2'}$); 159.57 ($C^{6'}$); 173.63 (CO_2H). RP HPLC: τ_R 12.1 min. LCMS (Supelcosil LC 18, 250×4.6 mm, 5 μm, MeCN–H_2O 85:15, flow rate 0.45 mL/min): τ_R 6.0 min; *m*/*z* (I_{rel}, %): 299 [M + H]$^+$ (100.0), 340 [M + MeCN + H]$^+$ (25.0). $C_{14}H_{14}N_6O_2$ (298.31). HRMS for $C_{14}H_{15}N_6O_2$ (M + H$^+$), calcd. 299.1256, found 299.1251.

***tert*-Butyl *N*-(2-acetylamino-9*H*-purin-6-yl)-(*R*)-valinate [(*R*)-10].** (*R*)-ValO*t*Bu×AcOH (0.47 g, 2.00 mmol) and TEA (0.37 mL, 2.67 mmol) were added to a suspension of compound **2** (141 mg, 0.67 mmol) in DMA (1.5 mL). The mixture was heated at 100 °C for 12 h, and then hot water (15 mL) was added. After cooling, the precipitate was separated by centrifugation, washed with water and dried *in vacuo*. Yellowish amorphous crude product was purified by flash column chromatography (eluent $CHCl_3$-MeOH from 10:0.05 to 10:0.20) to yield 139 mg (60%) of compound (*R*)-**10** as pale yellow crystals. Mp 124–126 °C. $[\alpha]_D^{20}$ +49.4 (*c* 1.0, $CHCl_3$). Anal. calcd. for $C_{16}H_{24}N_6O_3$: C, 55.16; H, 6.94; N, 24.12. Found: C, 54.98; H, 7.01; N, 24.03. ^{1}H NMR (DMSO-d_6, 100 °C) δ: 0.99 (d, 3H, CH_3-Val, *J* 6.8 Hz); 1.01 (d, 3H, CH_3-Val, *J* 6.9 Hz); 1.42 (s, 9H, *t*Bu); 2.21-2.30 (m, C^{β}H-Val); 2.26 (s, 3H, CH_3CO); 4.77 (br s, 1H, C^{α}H-Val); 6.57 (br s, 1H, $C^{6'}$NH); 7.94 (s, 1H, $C^{8'}$H); 9.15 (s, 1H, NHAc); 12.5 (br s, 1H, $N^{9'}$H). RP HPLC: τ_R 16.0 min. LCMS (column Phenomenex Luna C18, 150×2.0 mm, 3 μm, eluent MeCN–H_2O 9:1, flow rate 0.30 mL/min): τ_R 1.6 min; *m*/*z* (I_{rel}, %): 349 $[M + H]^+$ (100.0), 371 $[M + Na]^+$ (63.3), 387 $[M + K]^+$ (6.0), 412 $[M + MeCN + Na]^+$ (18.1). $C_{16}H_{24}N_6O_3$ (348.41).

***N*-(2-Acetylamino-9*H*-purin-6-yl)-(*S*)-valine *tert*-butyl ester [(*S*)-10]** was synthesized similarly to compound (*R*)-**10**. Yield 56%. Pale yellow crystals. Mp 126–127 °C. $[\alpha]_D^{20}$ –45.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR and mass spectra, τ_R (RP HPLC) were identical to those for compound (*R*)-**10**.

***N*-(2-Amino-9*H*-purin-6-yl)-(*R*)-valine [(*R*)-11]** was synthesized as described for compound **9**. Yield 69%. Colourless solid. Mp 267–268 °C (decomp.). *Ee* 81 %. Chiral RP HPLC: τ_R 5.9 min. Anal. calcd. for $C_{10}H_{14}N_6O_2$: C, 47.99; H, 5.64; N, 33.58. Found: C, 47.70; H, 5.59; N, 33.39. ^{1}H NMR (DMSO-d_6) δ: 0.96 (d, 3H, CH_3-Val, *J* 6.8 Hz); 0.97 (d, 3H, CH_3-Val, *J* 6.7 Hz); 2.22 (m, C^{β}H-Val); 4.64 (br s, 1H, C^{α}H-Val); 5.86 (s, 2H, NH_2); 6.68 (br s, 1H, $C^{6'}$NH); 7.75 (s, 1H, $C^{8'}$H); 12.3 (br s, 2H, $N^{9'}$H and CO_2H). ^{13}C NMR (DMSO-d_6) δ: 18.29 (CH_3-Val); 19.12 (CH_3-Val); 30.15 (C^{β}H-Val); 57.61 (C^{α}H-Val); 112.15 bs ($C^{5'}$); 136.69 ($C^{8'}$); 152.68 and 153.72 ($C^{4'}$ and $C^{2'}$); 159.38 ($C^{6'}$); 173.66 (CO_2H).

***N*-(2-Amino-9*H*-purin-6-yl)-(*S*)-valine [(*S*)-11]** was synthesized as described for compound **9**. Yield 74%. Colourless solid. Mp 270–272 °C (decomp.). *Ee* 86 %. Chiral RP HPLC: τ_R 6.8 min. NMR spectra were identical to those for compound (*R*)-**11**.

References for Supporting Information

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