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High-Pressure Synthesis of Enantiomerically Pure C-6 Substituted Pyrazolo[3,4-*d*]pyrimidines

Sally-Ann Poulsen,[a] David J. Young[b] and Ronald J. Quinn[a,*]

[a]*AstraZeneca R&D Griffith University, Brisbane, 4111, Australia*
[b]*Faculty of Science, Griffith University, Brisbane, 4111, Australia*

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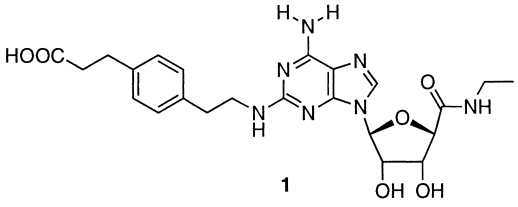
Abstract—The synthesis of enantiomerically pure C-6 substituted pyrazolo[3,4-*d*]pyrimidines has been performed by aromatic nucleophilic substitution of 4-amino-6-chloro-1-phenylpyrazolo[3,4-*d*]pyrimidine under conditions of high pressure at ambient temperature. Conventional synthetic conditions (reflux at atmospheric pressure) were unsuccessful. The *S* enantiomer **11** displayed higher affinity and selectivity for the adenosine A_1 receptor than the *R* enantiomer **12**.

Aromatic nucleophilic substitution at the C-2 position of the nucleoside analogue 2-chloroadenosine to give *N*-alkylated-2-aminoadenosines proceeds under relatively harsh conditions.[1–4] The general method is reaction of 2-chloroadenosine with an excess of amine at temperatures in excess of 100 °C for extended periods. Yields are variable and generally disappointing, however, the desirable biological properties of these compounds as adenosine A_{2A} receptor agonists has accounted for acceptance of these poor yields. The adenosine A_{2A} selective agonist CGS21680 (**1**) is a modified 2-substituted adenosine.[5]



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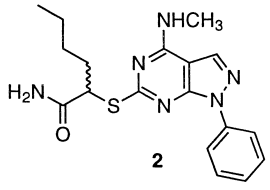

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Substitution at the corresponding position in phenylpyrazolo[3,4-*d*]pyrimidines resulted in α-[4-(methylamino)-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl]thiol]hexanamide (**2**) which has an A_1 K_i of 0.745 nM and is selective over the adenosine A_{2A} receptor.[6] Phenylpyrazolo[3,4-*d*]pyrimidines having potency and selectivity for the adenosine A_1 receptor have been synthesised following the general method as shown in Scheme 1. This synthetic scheme introduces the C-6 substituent via alkylation of sulfur in good yield. However, attempts in our laboratory to prepare C-6 substituted pyrazolo[3,4-*d*]pyrimidines with an enantiomerically pure centre α to the C-6 sulfur failed due to rapid racemisation, presumably due to carbanion stabilisation by both sulfur and the carbonyl. The corresponding compounds with a nitrogen at C-6 would be expected to be more stable.

4-Amino-6-chloro-1-phenylpyrazolo[3,4-*d*]pyrimidine **5** was synthesised via a two-step synthesis (Scheme 2). The one-pot reaction for formation of **4** from 4-amino-5-cyano-1-phenylpyrazole with benzoyl isocyanate proceeds through a urea intermediate, debenzoylation of this intermediate with base, followed by cyclisation.[7] 5-Amino-4-cyano-1-phenylpyrazole **3** (0.500 g, 2.7 mmol) was sealed under an atmosphere of argon. A solution of benzoyl isocyanate (0.600 g, 4.1 mmol) in 10 mL of dry DMF was injected into the reaction vessel and the reaction stirred for 12 h at 60 °C. Ammonium hydroxide (28%, 20 mL) was added to the reaction mixture, which was then stirred for a further 12 h at 60 °C. The solvent was removed under reduced pressure, the crude product was recrystallised from Me_2SO and water, affording pure **4**[8] in 73% yield. **4** (0.500 g, 2.20 mmol), phosphorous

*Corresponding author. Tel.: +61-7-3875-6000; fax: +61-7-3875-6001; e-mail: r.quinn@az.gu.edu.au

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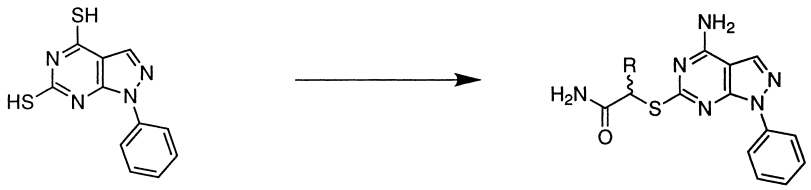
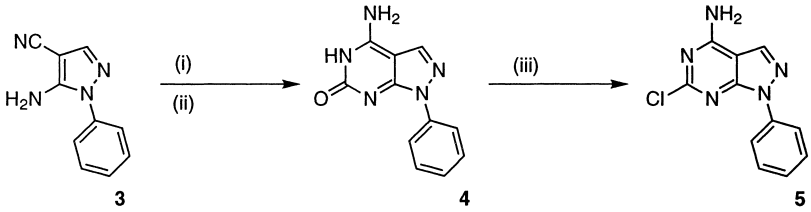
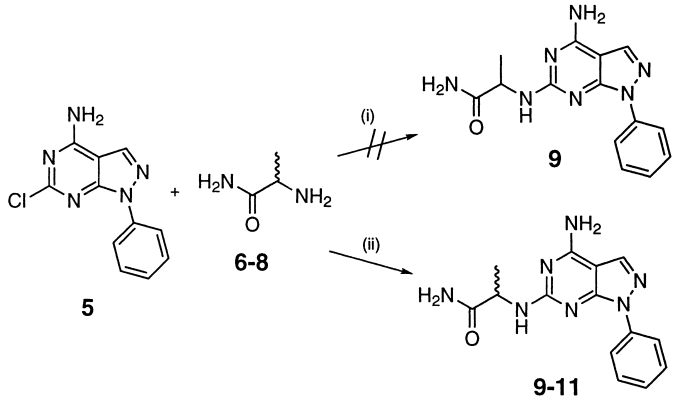


Scheme 1. General synthesis of 1-phenyl-4,6-disubstituted-pyrazolo[3,4-*d*]pyrimidines: (i) $BrCHRCONH_2$, py; (ii) CH_3I, NaOH (aq); (iii) NH_3 (g), EtOH, 110 °C, 72 h.



Scheme 2. Synthetic route to 4-amino-6-chloro-1-phenylpyrazolo[3,4-*d*]pyrimidine (**5**): (i) PhCONCO, DMF, 60 °C, 12 h; (ii) NH_4OH, 60 °C, 24 h, 73% overall; (iii) $POCl_3$, PCl_5, reflux, 40 h, 62%.



Scheme 3. Conventional and high-pressure synthetic methodology for target compounds **10**–**12**: (i) Conventional: DMF, DIPEA, reflux, 4 days; (ii) high pressure: 15 kbar, 40 °C, DMF, DIPEA, 7 days.

oxychloride (10 mL, 0.11 mol) and phosphorous pentachloride (2 g, 9.60 mmol) were refluxed for 40 h under an atmosphere of argon. Excess phosphorous oxychloride was removed by distillation under reduced pressure, leaving behind a yellow residue that solidified on cooling. Ice water (20 mL) was added to the solid residue with vigorous stirring. The aqueous solution was then extracted with diethyl ether (3 × 15 mL). The ether fractions were combined and dried over anhydrous $MgSO_4$, filtered, and the solvent removed, leaving a yellow solid. The crude product was recrystallised from Me_2SO and water giving **5**.[9]

Application of the conventional strategy to the nucleophilic aromatic substitution of the pyrimidine ring of 4-amino-6-chloro-1-phenylpyrazolo[3,4-*d*]pyrimidine (**6**) with 2-aminopropanamide (**7**) proved unsuccessful (Scheme 3). None of the substituted product was detected, even after 4 days of reaction. Synthesis at high pressure can now be carried out safely, and the method can be used for increasing the rates of many types of reactions, offering an alternative to synthesis at high temperatures.[10,11] In particular, synthesis at high pressure has been reported to increase the rate of reaction of nucleophilic aromatic substitutions, with neutral nucleophiles such as primary amines.[5] Compound **5** was reacted with 3 equiv of the appropriate 2-aminopropanamides, **6** (racemic), **7** (*S* enantiomer), or **8** (*R* enantiomer), in DMF with an equivalent of base under a pressure of 15 kbar at 40 °C for 7 days (Scheme 3).[12] The reaction mixtures were purified by chromatography to give the desired products **9** (racemic), **10** (*S* enantiomer) and **11** (*R* enantiomer) each in 68% yield. Unreacted starting material was the only other material recovered.

Table 1. Receptor binding results for rat A_1 and A_{2A} receptors[a]

Compd		A_1 receptor K_i (nM)	A_{2A} receptor K_i (nM)	A_{2A} K_i/A_1 K_i
9	Racemic	84.0±3.4	930±135	11
10	*S*	49.1±3.2	648±98	13
11	*R*	486±32	2120±339	4

[a]A_1 receptor binding data utilising competitive displacement of specific [^{3}H]-N^6-PIA binding from A_1 receptors in whole rat brain membranes. Data are the average of at least two independent experiments performed in duplicate and expressed as K_i±SEM. K_d of [^{3}H]-N^6-PIA was 1 nM. A_{2A} receptor binding data utilizing competitive displacement of specific [^{3}H]CGS21680 binding from A_{2A} receptors in rat striatal membranes. Data are the average of at least two independent experiments performed in duplicate and expressed as K_i±SEM. K_d of [^{3}H]CGS21680 was 14.9 nM.

These results demonstrate the utility of high pressure as an alternative to the use of elevated temperatures. Reaction under high pressure has made available C-6 amino substituted pyrazolo[3,4-*d*]pyrimidines and may offer a more facile route to 2-substituted purines than current methods. The results presented in Table 1 clearly highlight the stereochemical importance of the C-6 substituent for affinity to adenosine receptors. The *S* enantoimer **11** had 10-fold higher affinity for the A_1 receptor and 3.3-fold higher affinity for the A_{2A} receptor compared to the *R* enantiomer **12**. The *S* enantiomer was also more A_1 selective (13-fold) than the less potent *R* enantiomer (4-fold A_1 selectivity).

Acknowledgements

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8. **4** (Yield 73%); mp >350 °C ; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 7.27–8.15 (m, 5H, H-4′, H-3′, H-5′, H-2′, H-6′), 7.42 (br s, 1H, NH), 8.17 (s, 1H, H-3), 8.65 (br s, 1H, NH), 10.88 (br s, 1H, NH); ^{13}C NMR (50 MHz, Me_2SO-d_6) δ 92.6 (C-3a), 120.4 (C-2′, C-6′), 125.5 (C-4′), 128.9 (C-3′, C-5′), 135.6 (C3), 139.1 (C-1′), 153.6 (C-4), 156.2 (C-7a), 157.6 (C-6); IR (KBr disc) 3330 (1° amine NH); 3113 ($CH_{aromatic}$); 1670 cm^{-1} (C=O).
9. **5** (Yield 62%); ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 7.39–8.07 (m, 5H, H-4′, H-3′, H-5′, H-2′, H-6′), 8.35 (br s, 1H, NH), 8.38 (s, 1H, H-3), 8.49 (br s, 1H, NH); ^{13}C NMR (50 MHz, Me_2SO-d_6) δ 100.4 (C-3a), 121.0 (C-2′, C-6′), 126.7 (C-4′), 129.3 (C-3′, C-5′), 134.4 (C3), 138.4 (C-1′), 153.9 (C-7a), 158.1 (C-4 or C-6), 158.8 (C-4 or C-6); IR (KBr disc) 3441, 3298 (1° amine NH); 3114 cm^{-1} ($CH_{aromatic}$); ESMS (PI) 246.17. Calcd for (M + 1[H]); ESMS (NI) 244.16. Calcd for (M–1[H]).
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12. Experimental. **5** (0.050 g, 0.21 mmol) and each of **6**–**8** (0.060 g, 0.68 mmol) were dissolved in dry DMF (1.6 mL, 0.57 mmol). *N,N*-Diisopropylethylamine (0.1 mL) was added and the reaction mixtures transferred to 2 mL polyethylene bulbs. The bulbs were sealed with a brass clamp (excluding most of the air) and placed inside a Teflon reaction vessel, which was then filled with a mixture of castor oil/methanol (85%:15%). The reaction vessel was placed in a PSIKA Pressure Systems Limited Piston Cylinder High Pressure Reactor and the reaction carried out at 15 kbar, 40 °C for 7 days. Purification by chromatography (95% ethyl acetate/5% methanol) gave yields of 68%. In addition to standard characterisation, circular dichroism spectral data was recorded over a wavelength range of 350–200 nm. The racemic compound **9** lacked optical activity, while **10** and **11** exhibited optical activity, with measured values of $[\theta]_{226} = +3470$, $[\theta]_{245} = -1248$ and $[\theta]_{226} = -2961$, $[\theta]_{245} = +1006$, respectively. **10**: mp 163.7–164.7 °C; CD $[\theta]_{226}$ +3470, $[\theta]_{245}$ −1248; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.35 (d, 3H, $J = 7.1$ Hz, CH_3), 4.33 (m, 1H, CH), 6.69 (d, 1H, $J = 7.1$ Hz, 2° amine NH), 6.97 (br s, 1H, NH_{amide}), 7.25 (m, 1H, H-4′), 7.35 (br m, 3H, $NH_{amide, NH2}$), 7.48 (m, 2H, H-3′, H-5′), 8.10 (s, 1H, H-3), 8.29 (d, 2H, $J = 8.0$ Hz, H-2′, H-6′); ^{13}C NMR (50 MHz, Me_2SO-d_6) δ 18.5 (CH_3), 50.5 (CH), 96.4 (C-3a), 119.5 (C-2′, C-6′), 124.9 (C-4′), 128.9 (C-3′, C-5′), 134.2 (C3), 139.7 (C-1′), 155.9 (C-7a), 158.2 (C-4), 161.2 (C-6), 175.7 (C=O); IR (KBr disc) 3397 (1° amine NH); 3341, 3186 (1° amide NH); 1669 cm^{-1} (C=O); HRMS (EI) 298.1425. Calcd for $C_{14}H_{15}N_7O.H^+$: 298.1416. (EI) 298.1425. calcd for $C_{14}H_{15}N_7O.H^+$: 298.1416.