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A practical synthesis of the major 3-hydroxy-2-pyrrolidinone metabolite of a potent CDK2/cyclin A inhibitor

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Abstract—The synthesis of the major metabolite of a potent 3-aminopyrazole CDK2/cyclin A inhibitor is presented. A stereoconservative approach starting from malic acid was employed to construct the hydroxy-substituted pyrrolidinone moiety. In the key step of the synthesis the use of cyanoborohydride immobilized on Amberlyst 26 in trifluoroethanol represented a valid alternative to conventional solution-phase reducing agents.

CDKs/cyclins, a series of binary protein complexes showing genetic defects in many malignant diseases, are rate-limiting enzymes in cell cycle progression and as such represent excellent molecular targets for therapeutic intervention.[1,2] As part of our medicinal chemistry programme aimed at finding novel CDK2/cyclin A inhibitors, PHA-533533 (**1**, Fig. 1) was identified as a potential clinical candidate.[3] Compound **1** showed potent CDK2 inhibition in biochemical and cellular assays and also possessed, in comparison to other members of the same class, improved physico-chemical properties such as solubility and plasma protein binding.

The metabolic stability of **1** was investigated using both microsomes from different species (mouse, rat, dog, monkey and human) as well as rat hepatocytes. The major metabolite in all species (both hepatocytes and microsomes), except for mouse, was the mono-oxidized compound **2**. Another mono-oxidation product **3** was also identified in all species. In the mouse the ratio of **2** to **3** was reversed. A further minor metabolite **4** was also detected and is thought to arise from dehydration

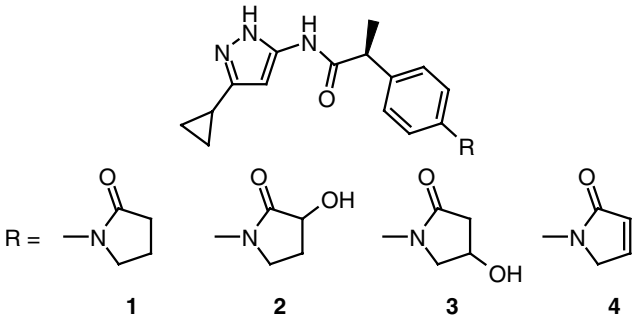

Figure 1. Structures of PHA-533533 (**1**) and metabolites.

of either **2** or **3**. This metabolite was only found in mouse microsomes where the metabolic rate of the compound **1** was very low (intr. CL <6 mL/min/kg). Elucidation of the structures of **2** and **3** was based upon the MS^n and accurate mass MS/MS spectra (Fig. 2).[4]

Hydroxy-substituted pyrrolidinones are components of many natural products and syntheses of the racemic core as well as enzymatic and stereoconservative preparations have been described.[5] In this communication, we report a short and practical synthesis of metabolite **2** (Scheme 1) via reductive alkylation of amine **5** with aldehyde **6**, prepared in turn from malic acid. Since the stereochemistry of the hydroxy group of metabolite **2** was unknown, the synthesis of both possible diastereomers,

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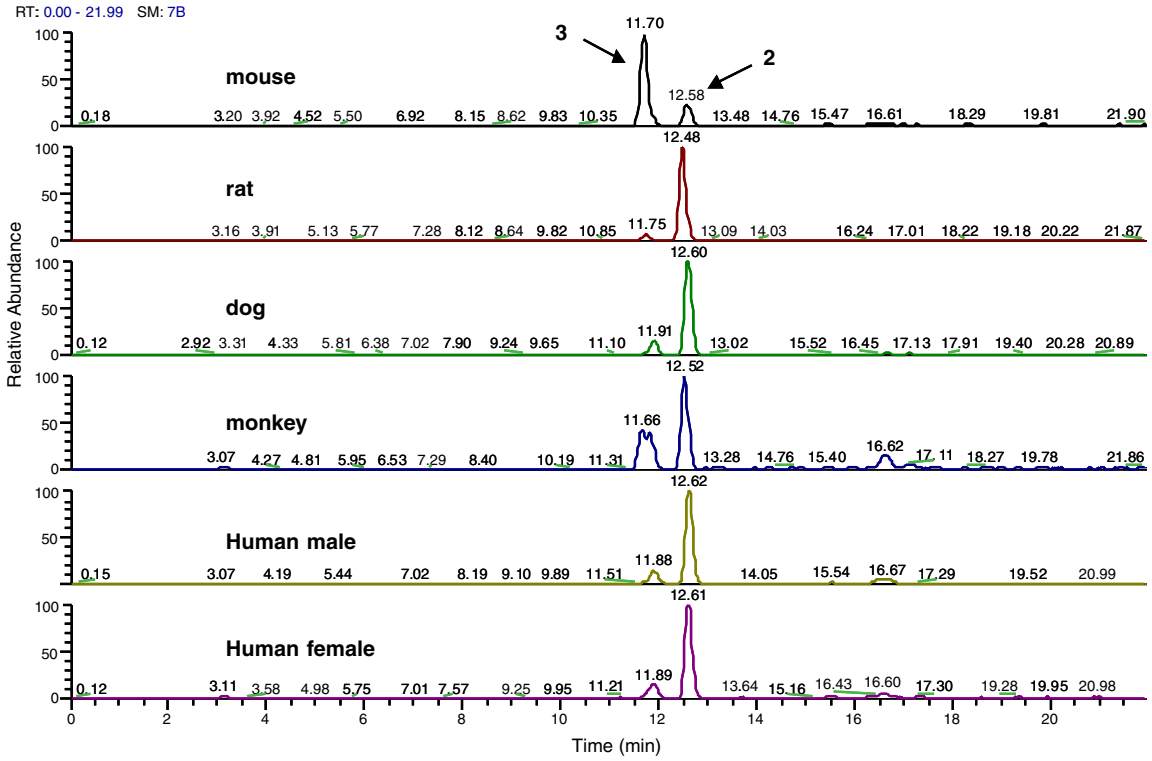
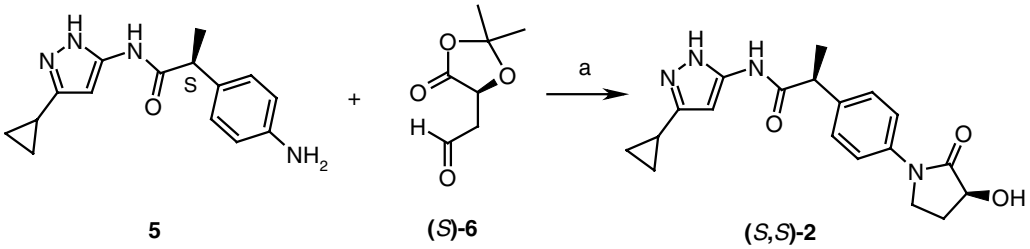


Figure 2. Cross species mass chromatograms at *m/z* 355 showing the ratio of metabolites **2** to **3** in microsomes incubated with **1**.



Scheme 1. Reagents and condition: (a) (polystyrylmethyl)trimethylammonium cyanoborohydride, TFE, rt, 12 h, 70%.

namely (*S*,*S*)-**2** and (*S*,*R*)-**2**, was performed starting from either enantiomer of malic acid.

The amino component **5** was prepared by reacting *tert*-butyl-5-amino-3-cyclopropyl-1*H*-pyrazole-1-carboxylate with (*S*)-2-(4-*tert*-butoxycarbonylamino-phenyl)-propionic acid chloride followed by removal of the protecting groups in 49% overall yield.[6b]

By a known three-step procedure[7] (*S*)-malic acid was protected as the acetonide (2,2-dimethoxypropane and *p*-TsOH) to give (*S*)-(2,2-dimethyl-1,3-dioxolan-4-one)-5-ethanoic acid in 95% yield. The carboxylic acid was then reduced to the alcohol (BH_3–THF) in quantitative yield and oxidized (PCC and DCM) to the corresponding aldehyde (*S*)-**6** (43% yield).

Reductive amination was performed at first with $Na(CN)BH_3$ in MeOH.[8] Under these conditions (rt, 5 h) formation of the secondary amine followed by spontaneous ring closure to produce the 3-hydroxy-2-pyrrolidinone derivative (*S*,*S*)-**2** did occur, but yields were low (~10%). The choice of the dioxolanone protecting group proved most convenient as it turned out to be more reactive towards nucleophilic displacement than an ordinary ester (methyl and ethyl). This enhanced reactivity allowed the spontaneous formation of the pyrrolidinone ring. Unfortunately, the presence of a by-product deriving from the attack of the dioxolanone intermediate by methanol was also observed.[9] In addition, the final product (*S*,*S*)-**2** resulted rather hydrophilic and therefore its separation from the contaminating excess of reducing agent proved rather tedious.

To overcome part of these problems, we tried $Na(CN)BH_3$ in trifluoroethanol for its less nucleophilic character but unfortunately the reaction was too slow.[10] We reasoned that solubility of the reagent under these conditions was not sufficient to ensure good reactivity and therefore we considered the use of cyanoborohydride immobilized on Amberlyst 26 as a possible alternative. Indeed, when amine **5** and aldehyde (*S*)-**6** were stirred together in trifluoroethanol in the presence of polymer-supported cyanoborohydride, not only clean

Table 1. CDK2/cyclin A inhibition and in vitro antiproliferative activity of PHA-533533 **1**, (*S*,*S*)-**2** and (*S*,*R*)-**2** metabolites

Compound	CDK2/cyclin A[a] IC_{50} (nM)[b]	A2780 cells[a] IC_{50} (μM)
1	37	0.8
(*S*,*S*)-**2**	27	2.2
(*S*,*R*)-**2**	27	1.6

[a] See Ref. 3a for description of biological assays.

[b] At least two independent experiments were performed for each compound in order to determine IC_{50} values. Potency is expressed as the mean of IC_{50} values obtained by non-linear least-squares regression fitting of the data. The coefficient of variation of the mean ranges from 10% to 24%.

formation of the secondary amine was observed but also subsequent ring closure to produce the desired lactam occurred very smoothly under the same reaction conditions employed for the reductive amination.[11] Moreover, by using an immobilized reagent, aqueous workup of the reaction could be avoided and therefore isolation of the hydrophilic final product resulted less troublesome.[12]

Both diastereomers (*S*,*S*)-**2** and (*S*,*R*)-**2** were tested and resulted equipotent in inhibiting CDK2/cyclin A in vitro but displayed lower antiproliferative activity on A2780 ovarian tumour cell line when compared to compound **1** (Table 1).

In summary, the major metabolite of a potent 3-aminopyrazole CDK2/cyclin A inhibitor was synthesized. By employing a stereoconservative approach, the two possible diastereomers were accessible starting from malic acid derivatives with opposite configuration.[13] Key step of the synthesis was the use of polymer-supported cyanoborohydride in trifluoroethanol as a valid alternative to conventional solution-phase reducing agents.

Acknowledgment

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References and notes

1. Sherr, C. J. *Science* **1996**, *274*, 1672.
2. For recent reviews, see: Huwe, A.; Mazitschek, R.; Giannis, A. *Angew. Chem., Int. Ed.* **2003**, *42*, 2122; Kong, N.; Fotouhi, N.; Wovkulich, P. M.; Roberts, J. *Drugs Future* **2003**, *28*, 881.
3. (a) Pevarello, P.; Brasca, M. G.; Amici, R.; Orsini, P.; Traquandi, G.; Corti, L.; Piutti, C.; Sansonna, P.; Villa, M.; Pierce, B. S.; Pulici, M.; Giordano, P.; Martina, K.; Fritzen, E. L.; Nugent, R. A.; Casale, E.; Cameron, A.; Ciomei, M.; Roletto, F.; Isacchi, A.; Fogliatto, G.; Pesenti, E.; Pastori, W.; Marsiglio, A.; Leach, K. L.; Clare, P. M.; Fiorentini, F.; Varasi, M.; Vulpetti, A.; Warpehosky, M. A. *J. Med. Chem.* **2004**, *47*, 3367; (b) Pevarello, P.; Brasca, M. G.; Orsini, P.; Traquandi, G.; Longo, A.; Nesi, M.; Orzi, F.; Piutti, C.; Sansonna, P.; Varasi, M.; Cameron, A.; Vulpetti, A.; Roletto, F.; Alzani, R.; Ciomei, M.; Albanese, C.; Pastori, W.; Marsiglio, A.; Pesenti, E.; Fiorentini, F.; Bischoff, J. R.; Mercurio, C. *J. Med. Chem.* **2005**, *48*, 2944.
4. Rat hepatocyte incubation: the compounds were incubated at 10 and 50 μM in a suspension of rat hepatocytes of 1×10^6 cells/ml. Samples from control incubation, performed in the same media but without the hepatocytes to test for the chemical stability of the compound, were also analysed. Samples of the hepatocyte and control incubations were taken at $t = 0$ min (immediately after drug addition) and at $t = 60$ min for profiling. Microsomal incubation: compounds, at a concentration of 10 μM, were incubated with liver microsomal preparations from different species including human. Metabolism was started by the addition of NADPH. Microsomal protein content was 1 mg/ml and the incubation time was 60 min. Control samples comprised the incubation performed in the absence of NADPH, and the incubation of the compound analysed in the incubation buffer in the absence of the microsomal fraction. Sample preparation: the hepatocyte samples, after centrifugation of small aliquots of the supernatant, were diluted with the same amount of aqueous mobile phase (A) to improve chromatography and were injected into the LC/MS system under the analytical conditions described below; the microsomal samples were injected 'as is'. Analytical methodology: an LCQ ion trap mass spectrometer equipped with electrospray ion source (ESI) and a Waters Alliance solvent delivery system was used under the following analytical conditions: HPLC: Column: Waters Symmetry C8 5 μm, 2.1 × 50 mm kept at a temperature of 40 °C in a suitable column oven. A guard column was also used. Mobile phase: 0.010 M, pH 4.5, ammonium formate (A)/acetonitrile (B) at the following gradient elution:

Time (min)	% A	% B	Flow (ml/min)
0	90	10	0.2
0.2	90	10	0.2
20	50	50	0.2
22	50	50	0.2
25	90	10	0.2
28	90	10	0.2

ESI capillary temp 250 °C. All the other parameters were set in order to obtain the best sensitivity of the protonated molecular ion of the unchanged drug (using the automated Xcalibur procedures). MS/MS conditions: collision energy was adjusted for each compound in order to have the most informative MS/MS spectra. The Autosampler was maintained refrigerated at 4 °C.
5. For some recent examples of stereoconservative synthesis of 3-hydroxy-2-pyrrolidinones, see: (a) Singh, S.; Crossley, G.; Ghosal, S.; Lefievre, Y.; Pennington, M. W. *Tetrahedron Lett.* **2005**, *46*, 1419; (b) Huang, P. Q.; Zheng, X.; Wei, H. *Heterocycles* **2003**, *60*, 1833; (c) Pires, R.; Burger, K. *Tetrahedron* **1997**, *53*, 9213.
6. (a) Pevarello, P.; Orsini, P.; Traquandi, G.; Varasi, M.; Fritzen, E. L.; Warpehosky, M. A.; Pierce, B. S.; Brasca, M. G. International Patent WO 01/12189; (b) Pevarello, P.; Orsini, P.; Traquandi, G.; Brasca, M. G.; Amici, R.; Villa, M.; Piutti, C.; Varasi, M.; Longo, A International Patent WO 02/48114.
7. Dutton, F. E.; Lee, B. H. *Tetrahedron Lett.* **1998**, *39*, 5313.
8. Borch, R. F.; Berstein, M. D.; Dupont Durst, H. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
9. The by-product was (*S*)-4-{4-[(*S*)-1-(5-cyclopropyl-2*H*-pyrazol-3-ylcarbamoyl)-ethyl]-phenylamino}-2-hydroxy-butyric acid methyl ester. The reduction with $Na(CN)BH_3$ was also tried in THF but, besides being very slow, cyclization did not occur.

10. For an example of reductive amination using $Na(CN)BH_3$ in trifluoroethanol, see: Ting, P. C.; Lee, J. F.; Anthes, J. C.; Shih, N. Y.; Piwinski, J. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2333.
11. Compound (*S*)-**6** (158 mg, 1 mmol) and **5** (270 mg, 1 mmol) were dissolved in trifluoroethanol (20 mL), treated with 1 g of (polystyrylmethyl)trimethylammonium cyanoborohydride (Novabiochem, loading: 3.0–4.5 mmol g^{-1}) and stirred overnight at room temperature. The resin was filtered off and washed with MeOH. Evaporation of the filtrate followed by flash chromatography on silica gel (eluent: 95/5 DCM–MeOH) of the crude afforded compound (*S*,*S*)-**2** in 70% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57 (d, J = 8.7 Hz, 2H); 7.34 (d, J = 8.7 Hz, 2H); 6.11 (s, 1H); 5.68 (d, J = 5.9 Hz, 1H); 4.26 (m, 1H); 3.80 (q, J = 7.1 Hz, 1H); 3.67 (m, 2H); 2.37–1.81 (m, 2H); 1.81 (m, 1H); 1.33 (d, J = 7.1 Hz, 3H); 0.86–0.60 (m, 4H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (br s, 1H); 7.55 (d, J = 8.8 Hz, 2H); 7.48 (d, J = 8.8 Hz, 2H); 6.47 (s, 1H); 4.46 (dd, J = 10.7, 8.2 Hz, 1H); 3.97 (q, J = 7.0 Hz, 1H); 3.76–3.66 (m, 2H); 2.49–2.12 (m, 2H); 1.80 (m, 1H); 1.55 (d, J = 7.0 Hz, 3H); 0.93–0.73 (m, 4H). Starting from (*R*)-**6**, following an identical procedure, (*S*,*R*)-**2** was obtained in 45% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.57 (d, J = 8.7 Hz, 2H); 7.33 (d, J = 8.7 Hz, 2H); 6.11 (s, 1H); 5.68 (d, J = 5.91 Hz, 1H), 4.26 (m, 1H); 3.80 (q, J = 6.9 Hz, 1H); 3.67 (m, 2H); 2.37–1.81 (m, 2H); 1.81 (m, 1H); 1.33 (d, J = 6.9 Hz, 3H); 0.85–0.60 (m, 4H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.69 (br s, 1H); 7.57 (d, J = 8.8 Hz, 2H); 7.48 (d, J = 8.8 Hz, 2H); 6.39 (s, 1H); 4.55 (t, J = 7.7 Hz, 1H); 3.88 (m, 2H); 3.75 (q, J = 7.5 Hz, 1H); 2.53–2.10 (m, 2H); 1.75 (m, 1H); 1.55 (d, J = 7.0 Hz, 3H); 0.90–0.68 (m, 4H).
12. For recent reviews on polymer-supported reagents, see: (a) Ley, S. V.; Baxendale, I. R. *Chem. Rev.* **2002**, *2*, 377; (b) Ley, S. V.; Baxendale, I. R.; Brusotti, G.; Caldarelli, M.; Massi, A.; Nesi, M. *Farmaco* **2002**, *57*, 321; (c) Kirschning, A.; Monenschein, R.; Wittenberg, R. *Angew. Chem., Int. Ed.* **2001**, *40*, 650; (d) Ley, S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, *23*, 3815.
13. We were not able to establish the correspondence between the authentic metabolite and the two synthetic diastereomers. From a chemical shift viewpoint the NMR spectra recorded in DMSO of both diastereomers and the metabolite were identical. Only the spectra exchanged with D_2O showed small differences in the coupling constants between the OH and the CH but not sufficiently in order to draw any definite conclusion. The differences in chemical shifts were evident only comparing the spectra in $CDCl_3$ of the two synthetic diastereomers. Unfortunately the isolated metabolite spectrum in $CDCl_3$ had not been recorded and the original sample was no longer available.