Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 2891–2894

TETRAHEDRON: *ASYMMETRY*

Synthesis and configuration of the cyclin-dependent kinase inhibitor roscovitine and its enantiomer

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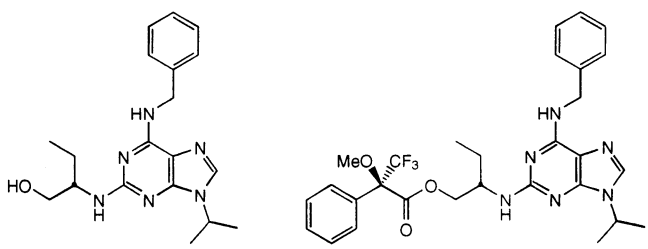
Received 22 October 2001; accepted 23 October 2001

Abstract—The cyclin-dependent kinase inhibitor (*R*)-2-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)butan-1-ol (roscovitine, **1a**), as well as its (*S*)-enantiomer **1b**, were synthesised. The chemical structure and absolute configuration of both enantiomers was confirmed by X-ray crystallography. Furthermore, high enantiomeric excess (>98%) was demonstrated by chiral chromatography of **1a** and **1b**, as well as NMR analysis of the diastereomeric Mosher's ester derivatives **2a** and **2b**.

1. Introduction

Active-site inhibitors of cyclin-dependent kinases (CDKs) have recently gained importance as potential therapeutics for the treatment of a range of proliferative disorders,[1] particularly cancer.[2] One compound class that has yielded many CDK-selective ATP antagonists is the 2,6,9-trisubstituted purines.[3,4] The representative of this compound class whose biological and pharmacological properties have been studied in most detail is known as roscovitine.[1,2,5] The synthesis of roscovitine† **1a** was first reported by Havlicek et al.,[6] who described this compound as (*R*)-2-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino) butan-1-ol (compound **26** in Ref. 6). There, **1a** was shown as the levorotatory enantiomer ($[\alpha]_D^{20} = -34.6$, *c* 0.43, $CHCl_3$), whereas the 2-(*S*)-enantiomer **1b** (compound **25** in Ref. 6) was found to be dextrorotatory ($[\alpha]_D^{20} = +35.3$, *c* 0.57, $CHCl_3$). Elsewhere,[7] **1a** was reported as having $[\alpha]_D^{20} = +35.1$, (*c* 0.29, $CHCl_3$), although the synthesis description was incorrectly entitled as the racemic modification. Another discrepancy arises from the melting points reported for the racemate **1** and the enantiomers **1a** and **1b**, i.e. 137–139, 102–104 and 118–120°C, respectively.[6] In order to reconcile these questions regarding the structure and stereochemistry of roscovitine, we decided to establish directly both absolute configuration and to demonstrate quantitatively the enantiomeric purity of roscovitine and its enantiomer.



1a (*R*)
b (*S*)

2a (*R*,*R*)
b (*R*,*S*)

2. Results and discussion

Using essentially the same synthesis methods as those described previously,[6] we found that several of our batches (chemical purity ≥95% by RP-HPLC) of roscovitine **1a** and its enantiomer **1b** consistently showed specific rotation values of opposite sign and

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† Also referred to as (*R*)-roscovitine, National Cancer Institute NSC No. 701554. Racemic material NSC No. 683246.

0957-4166/01/$ - see front matter
PII: S0957-4166(01)00471-2

significantly greater magnitude than previously reported,[6] i.e. $[\alpha]_D^{20}=+53\pm3$ and $[\alpha]_D^{20}=-53\pm3$ (c 0.56, $CHCl_3$), respectively. Furthermore, we found mp ranges of 106–108°C for all our batches, regardless of chirality, as would be expected.

As far as biological activity is concerned, roscovitine was reported to be two- to three-fold more potent than its (*S*)-enantiomer as an inhibitor of histone H1 phosphorylation by starfish $p34^{cdc2}$/cyclin B kinase,[8] while the racemate had intermediate activity.[6] In a different study, the same authors confirmed this result and showed the (*R*) absolute configuration of roscovitine through a crystal structure of roscovitine in complex with CDK2.[9] Working with recombinant human CDK2/cyclin E kinase (histone H1 phosphorylation, [ATP] = 0.1 mM),[10] we observed a three-fold potency difference, i.e. IC_{50} values of 80±20 nM and 240±20 nM for roscovitine and the (*S*)-enantiomer, respectively. This result is consistent with the relevant literature, although our IC_{50} value for roscovitine against CDK2/cyclin E is approximately 10-fold lower than that reported elsewhere, apparently using a similar assay.[11]

In order to establish the absolute configuration of roscovitine, we solved single-crystal structures of both enantiomers **1a** and **1b** by X-ray diffraction.[‡] In both structures, which are related to one another by inversion, there are two crystallographically independent molecules, which are essentially identical except for very minor differences in conformation. A plot of the structure of the (*R*)-isomer **1a** is shown in Fig. 1. Absolute structures of light-atom organic systems can normally be achieved by X-ray diffraction provided there is sufficient oxygen in the system to yield a measurable anomalous scattering signal with Cu-Kα radiation. In the case of the present system the oxygen content is only 4.5% by weight, this being comprised of a terminal oxygen atom exhibiting relatively high vibrational motion. Disorder in the isopropyl groups added further difficulties, and determination of the absolute structures of these molecules by conventional X-ray diffraction measurements with Cu-Kα radiation was not possible. Flack has shown that the precision of the Flack parameter (x) can be much improved by incorporating into the refinement a few very carefully measured data for selected enantio-sensitive reflections.[12] The value adopted by x is subject to systematic errors, for example absorption, which may drown out weak anomalous scattering effects. We have solved this problem by performing measurements at setting angles of 2θ, ω, χ and ϕ for one reflection and -2θ, $-\omega$, χ and ϕ for its Friedel opposite. Under these conditions the absorption factors for both are identical, and the quotient $F^2(\mathbf{h})/F^2(-\mathbf{h})$ is free from absorption (and extinction) errors. These quotients were measured for each of the eight Laue equivalents for a particular reflection, and the results averaged. Quotients which differed significantly from unity (7 for the (*R*)-isomer and 6 for the (*S*)-isomer) were incorporated into the refinement in the form of explicit restraints.[13] Even for this challenging case the Flack parameter adopted values of −0.05(5) and 0.00(6) for the (*R*)- and (*S*)-isomers, respectively. These values are well within the limits recently defined by Flack for strong enantio-distinguishing power.[14] Full details of this method will be published separately.

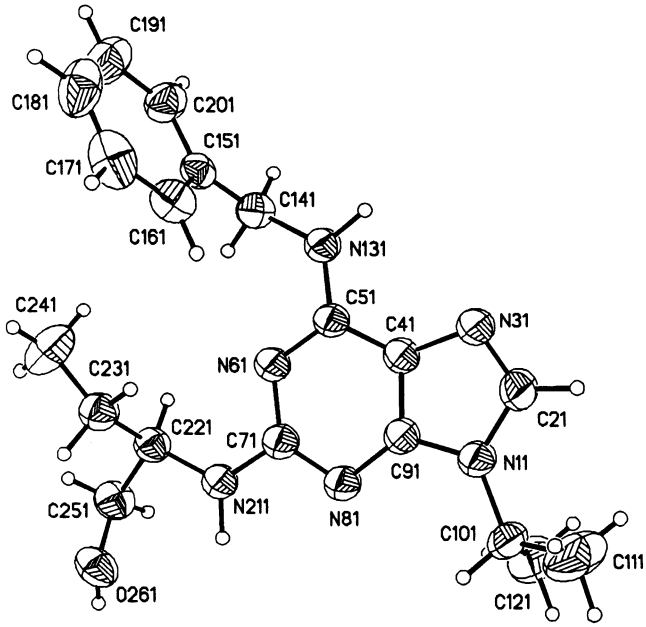

Figure 1. Molecular structure of **1a**; displacement ellipsoids enclose 50% probability surfaces. The minor disorder component about C101 has been omitted for clarity.

We also attempted to measure the enantiomeric purity of our preparations of **1a** and **1b**. Clean analytical separation of the enantiomers was achieved using chiral HPLC and an e.e. value of 98.3±1.8% was derived for roscovitine **1a**. Conversion of the enantiomers into diastereomeric esters using Mosher's method[15] was also performed. Derivatives **2a** and **2b** were prepared with the aid of highly enantiomerically pure (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride in the usual manner. Examination of the ^1H NMR spectra of **2** showed near-baseline separation of the multiplet signals arising from the methoxy protons of **2a** and **2b**, respectively. It was therefore possible to integrate these and to estimate e.e. values of ≥98% for both **2a** and **2b**, consistent with the chiral HPLC data. Separation of the diastereomeric CF_3 signals in the ^{19}F NMR spectra of **2** was also observed but resolution was not sufficient for quantitation purposes.

[‡] Crystallographic data (excluding structure factors) for both structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as deposition Nos. CCDC 157779 and 157780. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB2 1EZ UK (fax: +44 (1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk).

In conclusion, our data confirm conclusively that roscovitine **1a** does indeed possess the *R* absolute configuration but is the dextrorotatory enantiomer, whereas the *S* enantiomer **1b** is levorotatory. Furthermore, we demonstrate high enantiomeric purity for our preparations of **1a** and **1b**.

3. Experimental

3.1. General

Column chromatography:[16] Merck silica gel 60, 230–400 mesh. Optical rotation: Polaar 2001 (Optical Activity Ltd.) polarimeter. RP-HPLC: Vydac 201HS54 column, 4.6×250 mm, eluant A: 0.1% CF_3COOH in H_2O, B: 0.1% CF_3COOH in MeCN; flow rate 1 mL/min, 25°C. Mp: Reichert hot-stage microscope melting point apparatus; uncorrected. NMR spectra: Bruker DMX 500 NMR spectrometer; δ values in ppm rel. to $SiMe_3$ (^{1}H NMR) and $CFCl_3$ (^{19}F NMR; hexafluorobenzene was used as a secondary reference (−163 ppm)). Coupling constants *J* in Hz.

3.2. Synthesis of roscovitine enantiomers

(*R*)-2-(6-Benzylamino-9-isopropyl-9*H*-purin-2-ylamino) butan-1-ol **1a**. A mixture of 6-benzylamino-2-chloro-9-isopropyl-9*H*-purine, prepared as described,[6] (30.2 g, 0.1 mol) and excess (*R*)-(−)-2-amino-1-butanol (60 mL; $[\alpha]_D^{20}=-9.78$ (neat), 96% e.e./GLC) was heated at 160°C for 5 h in three sealed tubes with stirring. After cooling to room temperature, the combined reaction mixtures were diluted with $CHCl_3$ (450 mL), extracted with H_2O (3×210 mL), dried on $MgSO_4$, filtered and evaporated. The residue was purified by flash chromatography (120 g silica gel, 0–4% MeOH/CH_2Cl_2) and recrystallised from AcOEt/hexane to afford solid pale-green crude product (27.2 g, 77%). This material was re-chromatographed (120 g silica gel, AcOEt) twice and again recrystallised from AcOEt/hexane: colourless crystalline solid of **1a** (21.0 g, 59%). Mp 106–108°C. $[\alpha]_D^{20}=+56.3$ (*c* 0.56, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): 1.01 (t, $^3J=7.5$, 3H, CH_2CH_3); 1.51 (dd, $^3J=7.5$, 6H, $CH(CH_3)_2$); 1.55 (m, 1H, CH_2CH_3); 1.62 (m, 1H, CH_2CH_3); 3.61 (dd, $^2J=11$, $^3J=8$, 1H, CH_2OH); 3.80 (dd, $^2J=11$, $^3J=3$, 1H, CH_2OH); 3.88 (m, $^3J=8$, 6, 3, 1H, CHNH); 4.58 (hept., $^3J=7.5$, 1H, $CH(CH_3)_2$); 4.75 (bm, 2H, CH_2Ph); 4.87 (bd, $^3J=6$, 1H, CHN*H*); 5.09 (bs, 1H, O*H*); 6.09 (bs, 1H, N*H*); 7.23–7.36 (m, 5H, Ph); 7.44 (s, 1H, purine *H*(8)). ^{13}C NMR (125 MHz, $CDCl_3$): 10.91 (CH_2CH_3); 22.51, 22.58 ($CH(CH_3)_2$); 25.00 (CH_2CH_3); 44.42 (CH_2Ph); 46.42 ($(CH_3)_2CH_2$); 56.35 (CHNH); 68.51 (CH_2OH); 114.68 (purine *C*(5)); 127.34 (Ar-*C*H, *para*); 127.71, 128.58 (Ar-*C*H, *ortho*/*meta*); 134.58 (purine *C*H(8)); 138.71 (Ar-*C*); ~152 (purine *C*(4)); 154.81 (purine *C*(6)); 159.96 (purine *C*(2)); RP-HPLC: $t_R=17.3$ min (0–60% B in A over 20 min), $t_R=18.8$ min (23–33% B in A over 20 min), purity ≥99.5% (by integration at $\lambda=214$ nm). Anal. calcd for $C_{19}H_{26}N_6O$ (354.45): C, 64.38; H, 7.39; N, 23.71; found: C, 64.5; H, 7.3; N, 23.9.

(*S*)-2-(6-Benzylamino-9-isopropyl-9*H*-purin-2-ylamino) butan-1-ol **1b**. This was prepared in the same way as the (*R*)-enantiomer **1a** from 6-benzylamino-2-chloro-9-isopropyl-9*H*-purine with the exception that (*S*)-(+)-2-amino-1-butanol ($[\alpha]_D^{20}=+10.0$ (neat), 96% e.e./GLC) was used: colourless crystalline solid of **1b**. Mp 108–109°C (AcOEt/hexane), $[\alpha]_D^{20}=-56.3$ (*c* 0.56, $CHCl_3$). RP-HPLC: $t_R=17.3$ min (0–60% B in A over 20 min), $t_R=18.8$ min (23–33% B in A over 20 min), purity ≥99.5% (by integration at $\lambda=214$ nm); admixtures of **1a** and **1b** co-eluted. Spectroscopically **1b** was indistinguishable from **1a**.

3.3. Separation of enantiomers by chiral HPLC

A Chiralpak AD column (4.6×250 mm, 10 μm particles; # AD00CE-AK048, from Daicel Chemical Industries, Tokyo, Japan) was used. Isocratic elution with hexane/propan-2-ol (3:1, v/v) at 1 mL/min (20°C) was performed and quantitation was achieved by integration of UV-absorption ($\lambda=210$ nm) peaks. Samples were prepared at 1 mg/mL in MeOH and 10 μL aliquots were injected. $t_R=12.6$ min **1a**, 19.0 min **1b**. Limits of detection and quantitation were determined using admixture solutions of **1a** and **1b** in various proportions. The lower limit of quantitation was found to be 0.1%. For six different batches of **1a** e.e. = 98.3±1.8% (mean±standard deviation) was thus determined.

3.4. Crystal structure determinations

Single crystals of **1a** and **1b** were grown from AcOEt/iPr_2O. **1a** $C_{19}H_{26}N_6O$, $M=354.46$, orthorhombic, $a=11.4105(3)$, $b=15.4752(4)$, $c=22.4839(7)$ Å, $U=3970.20(19)$ Å^3, $T=220$ K, space group $P2_12_12_1$, $Z=8$, ((Cu-Kα) = 0.618 mm^{-1}. Refinement was performed against *F* with 5378 data with $F>4\sigma(F)$. The final *R*-factor was 3.72%. **1b** $C_{19}H_{26}N_6O$, $M=354.46$, orthorhombic, $a=11.4045(11)$, $b=15.4797(18)$, $c=22.489(3)$ Å, $U=3970.1(8)$ Å^3, $T=220$ K, space group $P2_12_12_1$, $Z=8$, ((Cu-Kα) = 0.618 mm^{-1}. Refinement was performed against *F* with 6010 data with $F>4\sigma(F)$. The final *R*-factor was 4.19%.

3.5. Synthesis and analysis of Mosher's ester derivatives

(*R*,*R*)-**2a** and (*R*,*S*)-3,3,3-Trifluoro-2-methoxy-2-phenylpropionic acid 2-(6-benzylamino-9-isopropyl-9*H*-purin-2-ylamino)butyl ester **2b**. Enantiomer **1a** or **1b** (50 mg, 0.14 mmol) was dissolved in $CDCl_3$ (1 mL). To the stirring solution was added (*R*)-(−)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride (54 μL, 73 mg, 0.29 mmol; ≥99% e.e./GLC). The reactions were complete after 5 min (TLC, 95:5 CH_2Cl_2/MeOH and ^{1}H NMR). The reaction mixtures were evaporated and the residues were purified by flash chromatography (3.5 g silica gel, 0–2% MeOH in CH_2Cl_2). The diastereomeric esters **2a** and **2b** were obtained as clear gums after evaporation and drying in vacuo (ca. 80%). ^{1}H NMR (500 MHz, $CDCl_3$) of admixtures or pure **2a** and **2b**: inter alii 3.50 (m, $J=0.64$, OCH_3, (*R*,*S*)); 3.52

(m, $J=0.85$, OCH_3, (R,R)). ^{19}F NMR (282.4 MHz, $CDCl_3$): −68.12 (s, (R,S)), −68.20 (s, (R,R)).

Acknowledgements

We thank Simon Olley and Craig McNair (Onyx Scientific Limited, Sunderland, England) for the chiral HPLC work and Dr John A. Parkinson (Department of Chemistry, University of Edinburgh, Scotland) for NMR spectroscopy. We also thank Dr. R. I. Cooper (University of Oxford) for modifications to the program CRYSTALS to enable the crystallographic restraints described here to be applied.

References

1. Meijer, L.; Leclerc, S.; Leost, M. *Pharmacol. Ther*. **1999**, *82*, 279–284.
2. Fischer, P. M.; Lane, D. P. *Curr. Med. Chem*. **2000**, *7*, 1213–1245.
3. Vesely, J.; Havlicek, L.; Strnad, M.; Blow, J. J.; Donella-Deana, A.; Pinna, L.; Letham, D. S.; Kato, J.; Detivaud, L.; Leclerc, S.; Meijer, L. *Eur. J. Biochem*. **1994**, *224*, 771–786.
4. Chang, Y. T.; Gray, N. S.; Rosania, G. R.; Sutherlin, D. P.; Kwon, S.; Norman, T. C.; Sarohia, R.; Leost, M.; Meijer, L.; Schultz, P. G. *Chem. Biol*. **1999**, *6*, 361–375.
5. Wang, D.; De la Fuente, C.; Deng, L.; Wang, L.; Zilberman, I.; Eadie, C.; Healey, M.; Stein, D.; Denny, T.; Harrison, L. E.; Meijer, L.; Kashanchi, F. *J. Virol*. **2001**, *75*, 7266–7279.
6. Havlicek, L.; Hanus, J.; Vesely, J.; Leclerc, S.; Meijer, L.; Shaw, G.; Strnad, M. *J. Med. Chem*. **1997**, *40*, 408–412.
7. Meijer, L.; Bisagni, E.; Legraverend, M. PCT Intl. Patent Appl. Publ. WO 97/20842, 1997; Chem. Abstr. 1997, 127, 104333.
8. Rialet, V.; Meijer, L. *Anticancer Res*. **1991**, *11*, 1581–1590.
9. De Azevedo, W. F.; Leclerc, S.; Meijer, L.; Havlicek, L.; Strnad, M.; Kim, S. H. *Eur. J. Biochem*. **1997**, *243*, 518–526.
10. Raynaud, F. I.; Nutley, B. P.; Goddard, P. M.; Kelland, L. R.; Valenti, M.; Brunton, L.; Eady, D.; Bell, G.; Marriage, H.; Fischer, P.; McClue, S.; Lane, D.; Workman, P. *Clinical Cancer Res*. **2000**, *6* (Suppl.), 317.
11. Meijer, L.; Borgne, A.; Mulner, O.; Chong, J. P.; Blow, J. J.; Inagaki, N.; Inagaki, M.; Delcros, J. G.; Moulinoux, J. P. *Eur. J. Biochem*. **1997**, *243*, 527–536.
12. Bernardinelli, G.; Flack, H. D. *Acta Crystallogr., Sect. A: Found. Crystallogr*. **1985**, *41*, 500–511.
13. Watkin, D. J.; Prout, C. K.; Carruthers, J. R.; Betteridge, P. W.; Cooper, R. I. *CRYSTALS*, Issue 11: Chemical Crystallography Laboratory, Oxford, UK, 2001.
14. Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr*. **2000**, *33*, 1143–1148.
15. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem*. **1969**, *34*, 2543–2549.
16. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem*. **1978**, *43*, 2923–2925.