Stable pyrano[2,3-*b*]quinoxalines and pyrano[2,3-*g*]pteridines related to molybdopterin

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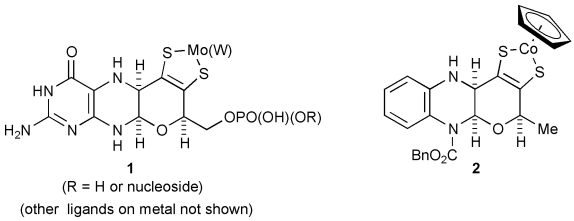
Received (in Cambridge, UK) 23rd October 2000, Accepted 15th November 2000
First published as an Advance Article on the web

The syntheses of the quinoxaline and pteridine proligands (3 and 4) related to molybdopterin are described and 3 has been characterised complexed *via* its dithiolene group to a CpCo(III) centre (5). Each of 3–5 has the sensitive hemiaminal unit of molybdopterin, unprotected, verifying its stability *in vitro*.

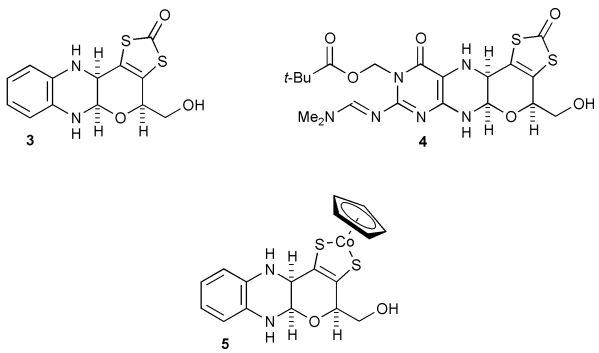
The pioneering studies on representatives of the molybdenum enzymes by Garrett and Rajagopalan[1] showed that each contained a prosthetic group that comprises a pteridine carrying a C_4-side-chain at pteridine C-6, having two sulfur atoms which ligate molybdenum. A series of X-ray crystallographic determinations on molybdenum and tungsten enzymes[2] further clarified the structure of the cofactor and its mode of ligation to Mo and W. Thus, the metals are chelated by an ene-1,2-dithiolate (dithiolene) which is part of a tricyclic system, in which a



dihydropyran ring is fused to a partially reduced pteridine, **1**. The pterin (2-aminopteridin-4(3*H*)-one) moiety is generally known as molybdopterin.

We have previously described[3] the synthesis of CpCo(III) (where Cp = η^5-cyclopentadienyl) complex **2** which involves ligation of the metal by a dithiolene moiety as part of a dihydropyran fused to a partially reduced pyrazine, as in **1**. However, **2** differs from **1** in several key respects. Firstly, **2** has a benzene ring instead of a pyrimidine ring; secondly the terminal primary hydroxy (phosphate) is absent; and thirdly, and crucially it retains a urethane at the nitrogen of the hemiaminal unit. It was far from certain that, freed from this protection against possible cleavage of the N–C–O system, the cyclic aminal would be stable, especially during the process of ligand release and complexation to a metal centre. Thus, to assess whether there are any special effects operating in the natural system which maintain the hemiaminal linkage, it was essential that structural analogues of **1** without the urethane be prepared. We describe here the synthesis of just such model compounds, the proligands **3** and **4** and the cobalt complex **5** derived from **3**.

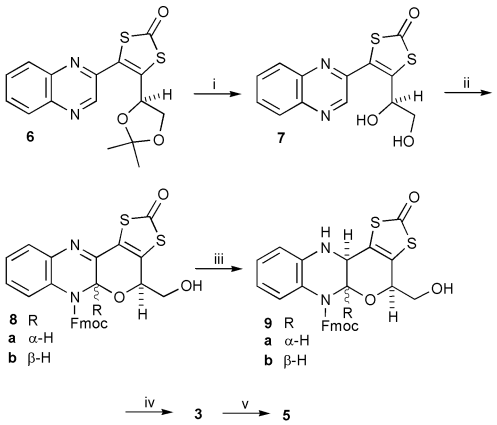
We have previously described syntheses of quinoxaline **6**[4] and protected pteridine **10**.[5] Hydrolysis of the acetal in **6** then reaction of **7** with fluoren-9-ylmethyl chloroformate (Fmoc-Cl), under carefully defined conditions gave a high yield of a 2:1 mixture of *cis*[6] (the desired) and *trans* tetracyclic products **8** which could not be separated by chromatography. Reduction of the mixture with sodium cyanoborohydride proceeded stereoselectively producing **9a**[6] and **9b**[6] which could be separated. It



is notable that there were no problems associated with the presence of a second alcohol group—neither cyclisation to give an oxepino[2,3-*b*]quinoxaline nor acylation of the primary hydroxy occurred.

Release of the Fmoc nitrogen protection, most efficiently using diethylamine,[7] gave the unprotected compound **3** *which retained the hemiaminal unit*. It remained to verify that this sensitive functionality would survive conditions to release the enedithiolate and form a metal complex. This indeed proved to be possible for, using caesium hydroxide for hydrolytic ligand release, with subsequent trapping by reaction with $CpCoI_2$, complex **5** was produced (Scheme 1).

A comparable sequence led to the isolation of a pyranopteridine having an unprotected hemiaminal unit. Thus, treatment of pteridine **10** with trifluoroacetic acid allowed selective removal of the acetal protection giving diol **11** then reaction of this with Fmoc-Cl produced a separable mixture of pyran-containing products in which the ratio of *cis*-**12a**[6] (desired) to *trans*-**12b**[6] (2:1) was comparable to that in the quinoxaline series. Conducting the cyclisation reaction at rt produced a more favourable ratio (4:1) of products but the reaction was much slower, not being complete after 1 week. *N*-Deprotection of the 'wrong' isomer **12b** led cleanly back to **11** (incidentally



Scheme 1 *Reagents and conditions*: i, $TsOH{\cdot}H_2O$, MeOH, reflux (92%); ii, Fmoc-Cl, 1,4-dioxane, 35 °C, 14 h (92%); iii, $NaB(CN)H_3$, AcOH, CH_2Cl_2, MeOH, rt (92%); iv, **9a**, Et_2NH, THF, rt (80%); v, CsOH, $CHCl_3$, MeOH, rt then $Cp(Co)I_2$ (55%).

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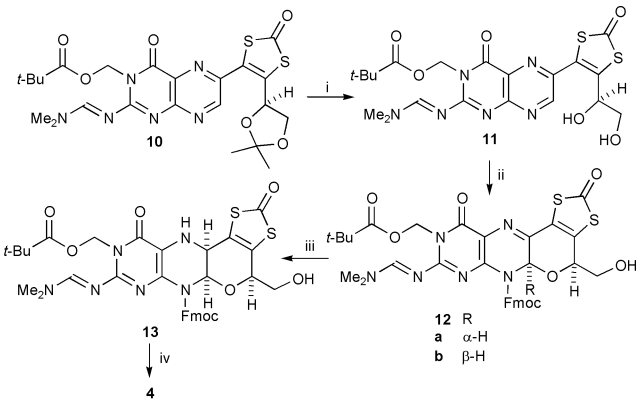
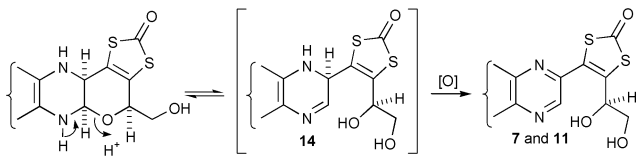
DOI: 10.1039/b008544h



Scheme 2 *Reagents and conditions*: i, TFA, CH_2Cl_2, 0 °C→rt (90%); ii, Fmoc-Cl, 1,4-dioxane, H_2O, 35 °C, 14 h (84%); iii, **11a**, $NaB(CN)H_3$, AcOH, CH_2Cl_2, MeOH, rt (92%); iv, Et_2NH, THF, rt (85%).

confirming the lability of the N–C–O unit) which could thus be recycled. Cyanoborohydride reduction of the *cis* isomer proceeded stereoselectively in high yield and removal of the N-8-protection from **13** gave pro-ligand **4**[6] with the hemiaminal unit intact and having the same relative stereochemistry[8] as the prosthetic groups in the natural cofactors (Scheme 2).

Both **3** and **4** are relatively stable compounds. However, after several weeks at rt, and without protection from moisture or oxygen, each reverted to the corresponding ring opened diol, **7** and **11**. This must involve reversible proton-catalysed cleavage of the N–C–O system revealing dihydro-systems **14** and then irreversible aerial oxidation (Scheme 3). The stabilities of the pyranoquinoxalines and pyranopteridine established in this work make it unlikely that any special properties of the enzyme environment need to be invoked to explain the tricyclic form of molybdopterin found in all the crystal structure determinations,[2] save that it must be protected from oxidation. The proton-catalysed cleavage of the N–C–O system, which we have



Scheme 3

suggested[9] may be intimately involved with catalysis at the metal centre, can now be studied *in vitro* with the compounds described herein. We shall be reporting on such studies in due course.

We thank the EPSRC for post-graduate (B. B.) and post-doctoral (B. B.) support for this work.

Notes and references

1 For a leading reference see R. M. Garrett and K. V. Rajagopalan, *J. Biol. Chem.*, 1996, **271**, 7387.

2 M. K. Chan, S. Mukund, A. Kletzin, M. W. W. Adams and D. C. Rees, *Science*, 1995, **267**, 1463; M. J. Romão, M. Archer, I. Moura, J. J. G. Moura, J. LeGall, E. Engh, M. Schneider, P. Hof and R. Huber, *Science*, 1995, **270**, 1170; H. Schindelin, C. Kisker, J. Hilton, K. V. Rajagopalan and D. C. Rees, *Science*, 1996, **272**, 1615; F. Schneider, J. Löwe, R. Huber, H. Schindelin, C. Kisker and J. Knäblein, *J. Mol. Biol.*, 1996, **263**, 53; R. Huber, P. Hof, R. O. Duarte, J. J. G. Moura, I. Moura, M.-Y. Liu. J. Legall, R. Hille, M. Archer and M. Romão, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 8846; J. C. Boyington, V. Sladishev, S. V. Khangulov, T. C. Stadtman and P. D. Sun, *Science*, 1997, **275**, 1305; J. Knäblein, H. Dobbeck, S. Ehlert and F. Schneider, *Biol. Chem.*, 1997, **378**, 293; C. Kisker, H. Schindelin, A. Pacheco, W. A. Wehbi, R. M. Garrett, K. V. Rajagopalan, J. H. Enemark and D. C. Rees, *Cell*, 1997, **91**, 973; M. Czjzek, J.-P. Dos Santos, J. Pommier, G. Giordano, V. Méjean and R. Haser, *J. Mol. Biol.*, 1998, **284**, 435; A. S. McAlpine, A. G. McEwan and S. Bailey, *J. Mol. Biol.*, 1998, **275**, 613; J. M. Dias, M. E. Than, A. Humm, R. Huber, G. P. Bourenkov, H. D. Bartunik, S. Bursakov, J. Calvete, J. Caldeira, C. Carneiro, J. J. G. Moura, I. Moura and M. J. Romão, *Structure*, 1999, **7**, 65; H. Dobbek, L. Gremer, O. Meyer and R. Huber, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 8884; H.-K. Li, C. Temple, K. V. Rajagopalan and H. Schindelin, *J. Am. Chem. Soc.*, 2000, **122**, 7673.

3 B. Bradshaw, A. Dinsmore, C. D. Garner and J. A. Joule, *Chem. Commun.*, 1998, 417.

4 A. Dinsmore, C. D. Garner and J. A. Joule, *Tetrahedron*, 1998, **54**, 3291

5 A. Dinsmore, C. D. Garner and J. A. Joule, *Tetrahedron*, 1998, **54**, 9559

6 In each case, relative stereochemistry was determined by NOE experiments involving the hydrogen atoms at the pyran/pyrazine ring junction and at the hydroxymethyl-bearing carbon.

7 M. Ueki, N. Nishigaki, H. Aoki, T. Tsurusaki and T. Katoh, *Chem. Lett.*, 1993, 721; K. C. Nicolau, C. W. Hummel, M. Nakada, K. Shibayama, E. N. Pitsinos, H. Saimoto, Y. Mizuno, K. Baldenuius and A. L. Smith, *J. Am. Chem. Soc.*, 1993, **115**, 7625.

8 D. C. Rees, Y. Hu, C. Kisker and H. Schindelin, *J. Chem. Soc., Dalton Trans.*, 1997, 3909.

9 S. P. Greatbanks, I. H. Hillier, C. D. Garner and J. A. Joule, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1529.