Tetrahedron 70 (2014) 7221–7228

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Synthesis of homochiral tetrahydropteridines

Stephen J. Baker [†], Kenneth J.M. Beresford [‡], Douglas W. Young [*]

Department of Chemistry, University of Sussex, Falmer, Brighton, East Sussex BN1 9QJ, UK

A R T I C L E I N F O

Article history:
Received 23 April 2014
Received in revised form 11 June 2014
Accepted 13 June 2014
Available online 18 June 2014

Dedicated to the memory of Professor Sandy McKillop

Keywords:
Tetrahydropteridines
Stereospecific
Synthesis
Homochiral

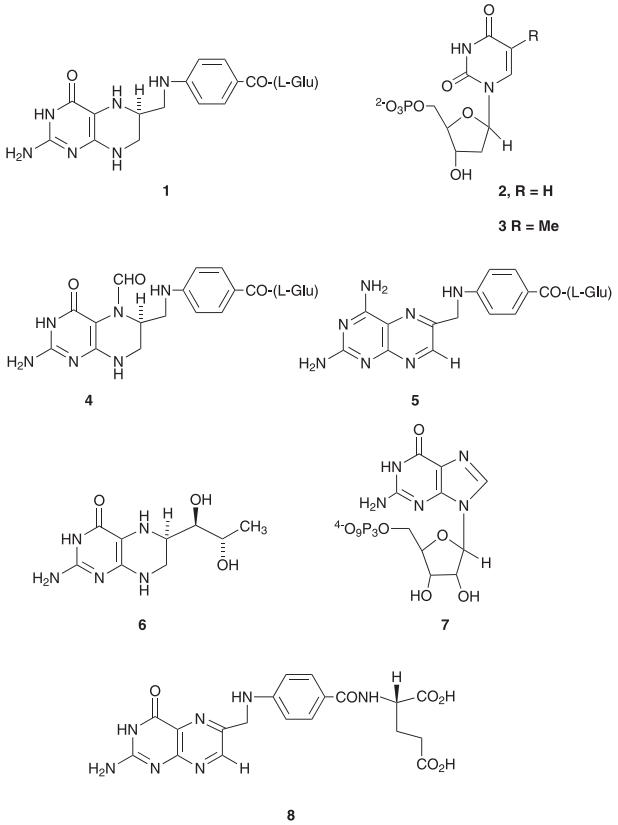
A B S T R A C T

A synthesis of protected homochiral tetrahydropteridines from (2*S*)-malic acid has been developed. This presents methodology for the synthesis of reduced pteridine coenzymes and pharmaceuticals.

1. Introduction

The reduced pteridine coenzyme, tetrahydrofolic acid **1**, is important for mediating enzyme-catalysed one-carbon transfer reactions.[1] Its involvement in the one-carbon transfer catalysed by thymidylate synthase (EC 2.1.1.45), which converts deoxyuridine monophosphate **2** into thymidine monophosphate **3** in a process requiring the enzyme dihydrofolate reductase (EC 1.5.1.3) for coenzyme regeneration, makes it important in the design of anticancer chemotherapeutics. The cancer rescue agent folinic acid **4**, which allows larger doses of the drug methotrexate **5** to be used in medicine is a one-carbon adduct of tetrahydrofolic acid.

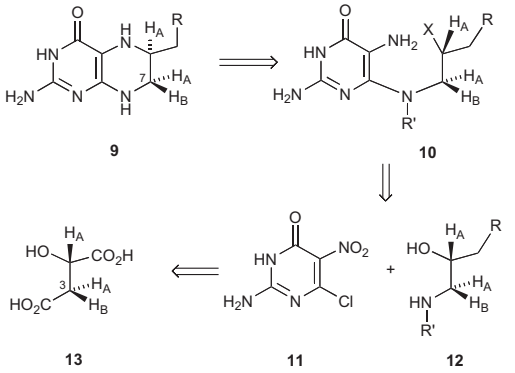
The coenzyme **1** and the related cofactor tetrahydrobiopterin **6**, which mediates enzyme-catalysed aromatic amino acid hydroxylation are biosynthesised from guanosine triphosphate (GTP) **7** in several enzyme-catalysed steps by microorganisms. Mammals, however, cannot synthesise tetrahydrofolate **1** by this route and require to take the vitamin folic acid **8** in their diet, reducing it to the coenzyme using dihydrofolate reductase. This makes the enzymes involved in the microbiological synthesis targets for antibacterial drugs.



* Corresponding author. E-mail address: d.w.young@sussex.ac.uk (D.W. Young).
† Present address: GlaxoSmithKline, 1250 S. Collegeville Road, Collegeville, PA, 19426-0989, USA.
‡ Present address: Leicester School of Pharmacy, Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester, LE1 9BH, UK.

http://dx.doi.org/10.1016/j.tet.2014.06.048
0040-4020/

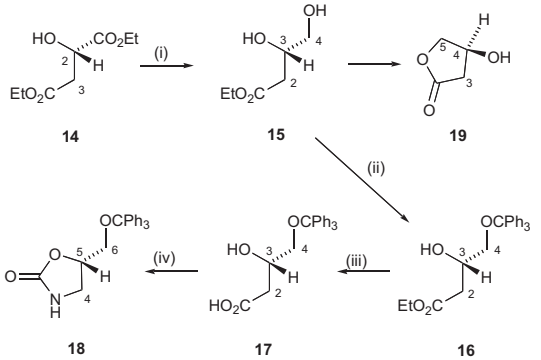
Most homochiral reduced pteridines have so far been accessed by semi-synthetic or biological methods and we now wish to report a totally synthetic method, which will allow access to a variety of targets containing the natural stereochemistry at C-6. Our retrosynthetic plan, suggested in Scheme 1, requires inversion of stereochemistry in the step **9**⇒**10** to obtain the appropriate stereochemistry at C-6, and so (2*S*)-malic acid **13** was chosen as starting material. Since we had already developed methods for obtaining large quantities of samples of (2*S*)-malic acid, **13**, H_A=^{2}H and **13**, H_B=^{2}H, which are deuteriated stereospecifically at C-3,[2] the synthesis will also allow for the preparation of samples of the co-enzymes **1** and **6**, which are stereospecifically labelled at C-7 for studies of the Amadori rearrangement involved in their biosynthesis.



Scheme 1.

2. Results and discussion

Our first tasks were selectively to convert the α-carboxyl group of (2*S*)-malic acid **13** into a potential side chain and to convert the β-carboxyl group into an amine. Conversion of the α-carboxylate into a hydroxymethyl group has been achieved by Saito et al.[3] who selectively reduced the α-ester of diethyl (2*S*)-malate **14** in good yield and we used this reaction to obtain the diol **15** in 87% yield as shown in Scheme 2.



Scheme 2. Reagents and conditions: (i) Ref. 3; (ii) Ph_3CCl, pyridine, rt 1 h, then 50 °C, 4 h (95%); (iii) 1 N aq NaOH, THF, rt, 36 h (93%); (iv) (a) $ClCO_2^iBu$, Et_3N, THF, −30 °C, 1 h, (b) NaN_3, H_2O, 0 °C, 1.5 h, (c) toluene, 60 °C, 1 h, then reflux, 30 min (30%).

This compound spontaneously formed the lactone **19** on standing and so it was immediately converted into the trityl derivative **16** in 95% yield by reaction with triphenylmethyl chloride in pyridine. Hydrolysis using aqueous sodium hydroxide in tetrahydrofuran gave the acid **17**. This was converted into the mixed anhydride with *iso*-butyl chloroformate and subsequent reaction with sodium azide gave the corresponding azide. Heating resulted in Curtius rearrangement and cyclisation of the intermediate isocyanate to give the oxazolidinone **18**. Although we had prepared a useful synthetic intermediate for our target compound **12**, problems were encountered in scaling up and so the alternative route shown in Scheme 3 was developed.

α-Methyl (2*S*)-malate **20**, prepared using the method of Miller,[4] was reacted with diphenylphosphoryl azide and triethylamine. Curtius rearrangement of the resultant azide with spontaneous cyclisation of the intermediate isocyanate gave the oxazolidinone **21** in 74% yield. This was converted into the urethane **22** in 95% yield using di-*tert*-butyl dicarbonate, triethylamine and dimethylaminopyridine in dioxane and the ester was reduced using sodium borohydride in tetrahydrofuran at −15 °C to afford the alcohol **23**. Although this was converted into the *tert*-butyldiphenylsilyl ether **24** using *tert*-butyldiphenylsilyl chloride, DMAP and triethylamine in dichloromethane, various attempts to hydrolyse this directly to the compound **26** proved fruitless. The alcohol **23**, however, underwent cleavage using caesium carbonate in methanol at room temperature to afford the diol **25** and this was converted into the silyl ether **26** in 87% yield on reaction with *tert*-butyldiphenylsilyl chloride, triethylamine and DMAP in dichloromethane at room temperature. The urethane protecting group was now removed using trifluoroacetic acid and the resultant amine **27** was reacted in triethylamine and methanol with 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11**, prepared by the method of Wood,[5] to give the adduct **28**.

Various unsuccessful attempts were made to convert the product **28** to an amino-mesylate analogue of compound **10** (X=OMs), which might be induced to cyclise to the required reduced pteridine. Arguing that the free 6-amino moiety of **28** might form an aziridine intermediate, and encouraged by the fact that a benzylated analogue had been shown to cyclise,[6] we converted the amine **27** into the benzyl derivative **29** in 64% yield by reaction with benzaldehyde and triethylamine in ethanol followed by in situ reduction with sodium borohydride as shown in Scheme 4. Reaction with 2-amino-6-chloro-5-nitro-4-(3*H*)-pyrimidinone **11** then gave the product **30** in good yield.

The pyrimidine **30** was now converted into the triflate using triflic anhydride and pyridine and this was hydrogenated in tetrahydrofuran containing catalytic quantity of 10% palladium on carbon. The product displayed *m*/*z* (FAB, 3-NBA) 526, which was in keeping with $[M+H]^+$ for the desired compound **31** but the ^{1}H NMR spectrum was ill-resolved and the product showed a tendency to oxidise. We therefore repeated the ring-closure reaction but immediately treated the product **31** with freshly prepared[7] formic acetic anhydride. The product was purified by extensive HPLC in 19% yield and had the spectroscopic characteristics of the desired compound **32** containing a small amount of bis formylated material.

When the methoxymethylene protected compound **37** was prepared from the oxazolidinone **21** as outlined in Scheme 5 and described in the Experimental section, mesylation followed by hydrogenation using 10% palladium on charcoal in methanol gave a compound, which was reacted with freshly prepared[7] formic acetic anhydride in pyridine.

The spectra of the product indicated that it was the cyclised triformyltetrahydropteridine **38**, which interestingly, appeared to exist as the 4-phenol tautomer rather than the more usual 3,4-amide. In NOE experiments, summarised in Fig. 1, irradiation of the multiplet at δ 4.86 ppm for H-6 caused enhancement of the singlet at δ 8.70 ppm for the 5-formyl proton and of the peaks due to H-7 and H-9. Irradiation of the broad singlet at δ 12.1 ppm also caused enhancement of the singlet at δ 8.70 ppm for the 5-formyl proton. Irradiation of the singlet at δ 8.60 ppm for one 4-*N*-formyl