A Simple and Effective Method for Preparation of the 6(*R*)- and 6(*S*)-Diastereoisomers of 5-Formyltetrahydrofolate (Leucovorin)

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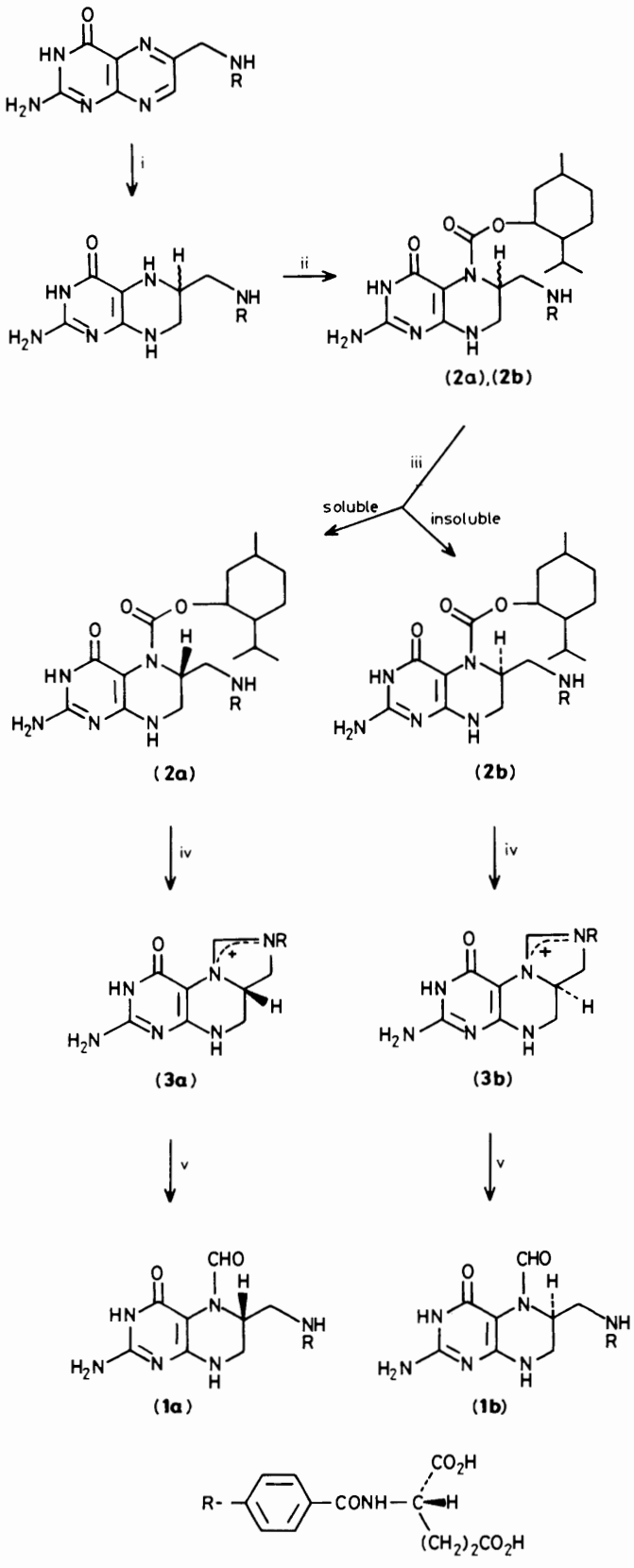
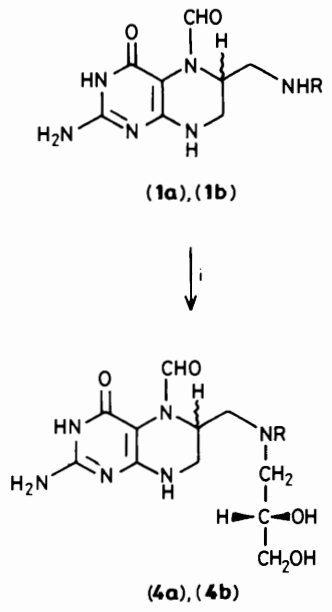
Acylation of 6(*RS*)-tetrahydrofolate with (−)-menthyl chloroformate afforded an N-5 derivative which was separable into its diastereoisomers by extraction with n-butanol; these derivatives were converted separately into the 6(*R*)- and 6(*S*)-diastereoisomers of 5-formyltetrahydrofolate by treatment with a mixture of formic acid and hydrogen bromide in acetic acid followed by hydrolysis.

5-Formyltetrahydrofolate (leucovorin) is widely used in rescue therapy of patients undergoing chemotherapy with anticancer agents such as methotrexate.[1] The role of leucovorin in such therapy is to bypass the inhibition of one carbon metabolism brought about by methotrexate. It is used as the 6(*RS*)-diastereoisomeric mixture (**1a**) + (**1b**) but only the natural 6(*S*)-diastereoisomer (**1b**) is effective.[2] The mixture of diastereoisomers has been separated by fractional crystallisation[3] and by chromatography[4] and the 6(*S*)-isomer has been prepared *via* stereospecific reduction of dihydrofolate catalysed by dihydrofolate reductase.[5] All of these methods are inconvenient for obtaining substantial quantities of the 6(*S*)-isomer for therapeutic use or for obtaining either single diastereoisomer for experimental use. The importance of such experiments is indicated by observations that the non-natural 6(*R*)-isomer *inhibits* some of the enzymes involved in one carbon transfer;[6] hence the natural 6(*S*)-isomer may have therapeutic advantages. We have found a rapid and effective method for preparing both the 6(*R*)- and 6(*S*)-diastereoisomers of leucovorin from folic acid in substantial quantity.[7]

Folic acid was reduced to tetrahydrofolate with sodium borohydride in aqueous sodium hydroxide (Scheme 1). After adjustment of the pH to 7, (−)-menthyl chloroformate was added to prepare the diastereoisomeric 5-(−)-menthyloxycarbonyl derivatives (**2a,b**) isolated after acidification of the reaction mixture. This mixture was shown to consist of equal quantities of two components by h.p.l.c. (octadecylsilyl SiO_2, 25:75 acetonitrile–tris HCl buffer, 50 mM, pH 7, containing mercaptoethanol, 10 mM). The mixture of the dry diastereoisomers (**2a,b**) was then stirred with dry n-butanol overnight and centrifuged to afford a soluble fraction I and an insoluble fraction II. H.p.l.c. analysis of these fractions showed that fraction I contained 77% of one diastereoisomer with 23% of the other whereas fraction II contained a mixture of 17% and 83% respectively of the diastereoisomers. It was subsequently shown that fraction I was enriched in the (*R*)-isomer and



Scheme 1. *Reagents:* i, $NaBH_4$, aq. NaOH; ii, (−)-menthyl chloroformate, pH 7; iii, butan-1-ol extraction; iv, HCO_2H, HBr in HOAc; v, pH 6.5—7.0 (reflux), then $CaCl_2$.



Scheme 2. Reagents: i, D-(+)-glyceraldehyde, $Na(CN)BH_3$, pH 6.

fraction II in the (*S*)-isomer (see below). Further partition of the enriched fractions with dry n-butanol led to samples containing about 95% of a single diastereoisomer. That a separation had been obtained was supported by the 250 MHz ^1H n.m.r. spectra (CD_3SOCD_3) which showed signals at δ 7.76, 7.79, 6.89, and 6.92, characteristic of the benzene ring protons of the (*R*)-isomer and δ 7.72, 7.76, 6.81, and 6.84 characteristic of the (*S*)-isomer. All peaks were present equally in the diastereoisomeric mixture (**2a,b**).

The (−)-menthyloxycarbonyl derivatives were separately converted into the 5,10-methenyltetrahydrofolates (**3a**) and (**3b**) using a mixture of formic acid and acetic acid saturated with hydrogen bromide at 55—60 °C and the products were isolated in the presence of mercaptoethanol to prevent oxidation. The salts (**3a**) and (**3b**) were then converted into the 5-formyl compounds (**1a**) and (**1b**) by hydrolysis (reflux) at pH 6.5—7.0 in the absence of air. The calcium salts were isolated. The products were identical by h.p.l.c. and n.m.r. spectroscopy with the commercial 6(*RS*)-diastereoisomer and the 6(*S*)-diastereoisomer prepared by us enzymically.[5]

In order to establish the chirality of the separated materials, it was necessary to prepare a further diastereoisomeric derivative (Scheme 2). This was achieved by treatment with D(+)-glyceraldehyde and sodium cyanoborohydride which afforded the N-10 dihydroxypropyl derivatives (**4a,b**). Although these compounds could not be separated by h.p.l.c., 250 MHz ^1H n.m.r. spectroscopy allowed the configurations to be assigned from the chemical shifts of the formyl protons. Thus the derivative of the enzymically prepared material showed a peak at δ 8.71 and that of the diastereoisomeric mixture showed peaks at δ 8.71 and 8.80. The signal at δ 8.71 thus characterised the 6(*S*)-isomer whereas the signal at δ 8.80 characterised the 6(*R*)-isomer. When the fractionated derivatives were examined in this way, that obtained from fraction I showed a signal at δ 8.80 and was thus shown to be the 6(*R*)-isomer. Conversely, the sample derived from fraction II showed a signal at δ 8.71 characteristic of the 6(*S*)-isomer.

The overall conversion of folic acid into a single diastereoisomer of 5-formyltetrahydrofolate was 40% and we have prepared 10—15 g of each diastereoisomer by this method.

These results show that both the 6(*R*)- and (6*S*)-diastereoisomers of 5-formyltetrahydrofolate can now be readily prepared on a substantial scale.

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