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CARBOXYLIC ACID BIOISOSTERES OF γ-LINKED DIPEPTIDE ANALOGUES OF THE FOLATE-BASED THYMIDYLATE SYNTHASE (TS) INHIBITOR, 2-DESAMINO-2-METHYL-N^{10}-PROPARGYL-5,8-DIDEAZAFOLIC ACID (ICI 198583)

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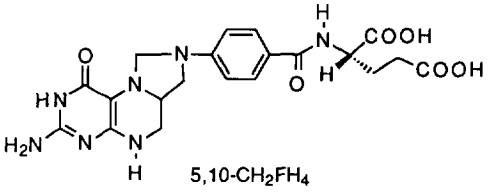
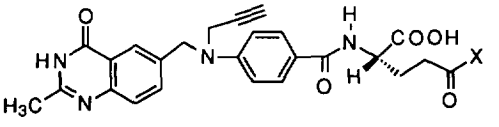
Abstract. Tetrazole carboxylic acid bioisosteres of γ-linked dipeptide derivatives of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583), a folate-based inhibitor of thymidylate synthase (TS), were synthesised by multistep routes starting from the appropriate pteroic acid analogue and Z-D-Ala or D-Glu. They exhibited excellent TS inhibitory activities which, however, were not accompanied by a parallel improvement in the L1210 cell growth inhibition.

Thymidylate synthase (TS) is an important target in the search for new anticancer agents since it catalyses the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), a nucleotide exclusively required for DNA synthesis. The one-carbon unit required for this reaction to occur is donated by the folate cofactor N^5, N^{10}-methylene-5,6,7,8-tetrahydrofolate (5,10-CH_2FH_4).[1]



5,10-CH_2FH_4



1 (ICI 198583), X = OH
2 (ICI 198583-γ-L-Ala), X = L-Ala
3 (ICI 198583-γ-D-Glu), X = D-Glu
4 (ICI 198583-γ-D-Ala), X = D-Ala

Over the last two decades many folate-based inhibitors of TS have been synthesised as anticancer agents. Although N^{10}-propargyl-5,8-dideazafolic acid[2] (CB 3717) had to be withdrawn from clinical trials because of undesirable renal toxicity,[3] Tomudex™ (ZD 1694),[4] an inhibitor of TS that undergoes polyglutamation,

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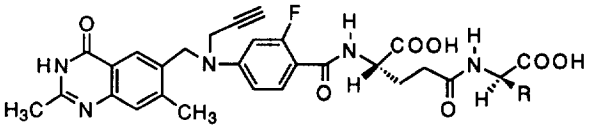
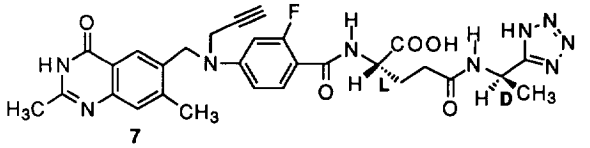
has recently completed Phase III clinical trials in which promising activity against colorectal cancer was observed.[5] Other antifolates (*e.g.* LY231514,[6] BW1843U89,[7] and AG 337[8]) directed against the TS enzyme are currently under clinical evaluation.



5 (7-Me, 2'-F ICI 198583-γ-D-Glu), R = CH_2CH_2COOH
6 (7-Me, 2'-F ICI 198583-γ-D-Ala), R = CH_3

In our search for compounds with a different spectrum of activity to Tomudex™, we were interested in synthesising potent inhibitors of TS which do not undergo polyglutamation. γ-Linked L, L dipeptide analogues of 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic acid (ICI 198583), *e.g.* ICI 198583-γ-L-Ala (**2**), were the first class of compounds synthesised in this series.[9] While these compounds showed excellent *in vitro* properties their utility *in vivo* as inhibitors of TS that do not undergo polyglutamation was limited because of degradation to the monoglutamate ICI 198583,[9] a compound shown to undergo polyglutamation. To overcome this problem, the second residue of γ-linked L, L dipeptide analogues of ICI 198583 was replaced by D-amino acids to give γ-linked L, D dipeptide analogues, *e.g.* ICI 198583-γ-D-Glu (**3**), ICI 198583-γ-D-Ala (**4**), which were shown to be potent inhibitors of TS, non-substrates for FPGS and resistant to *in vivo* enzymatic degradation.[10] Further enhancement in TS inhibition was achieved with the 7-Me, 2'-F derivative of ICI 198583-γ-D-Glu (**5**).[10] In an attempt to prepare even more potent inhibitors of TS, the replacement of the carboxyls of these dipeptides with acid mimics was explored. To test out this idea, tetrazole, one of the most widely used carboxylic acid isosteres,[11] was the initial acid mimic of choice. Compounds **7, 8,** and **9,** derivatives of **6, 3,** and **5** respectively, were therefore synthesised.



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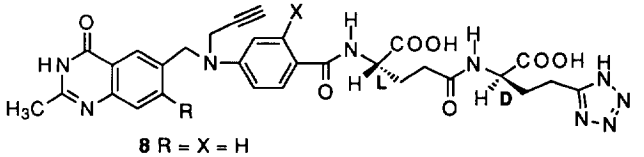
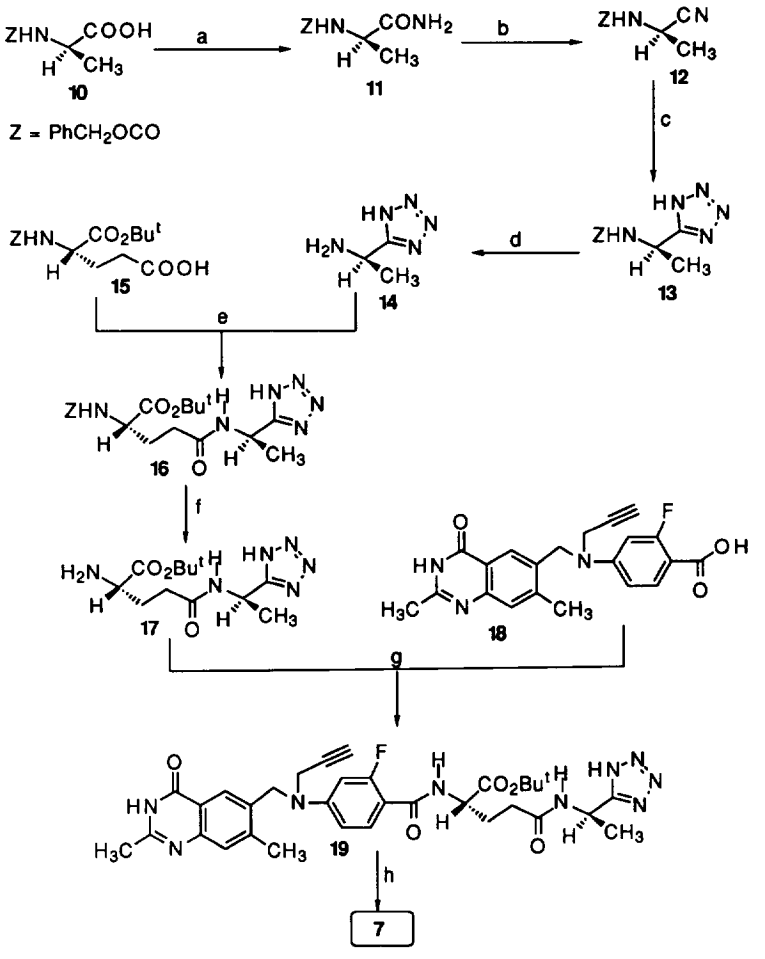


8 R = X = H
9 R = CH_3, X = F

The synthetic pathway to antifolate **7** is shown in Scheme 1. The initial synthetic target in this multistep sequence was compound **13** (Z-D-AlaT). This was prepared in 3 steps from **10** (Z-D-Ala) using a

modification of Grzonka's methodology,[12,13] and the optical rotation of +36.46° (c=1.15, MeOH) was virtually identical to that reported by Grzonka and Liberek (+34.5°; c=1, MeOH) who obtained optically pure Z-D-AlaT by resolving Z-DL-AlaT using L-tyrosine hydrazide as the resolving agent.[14]

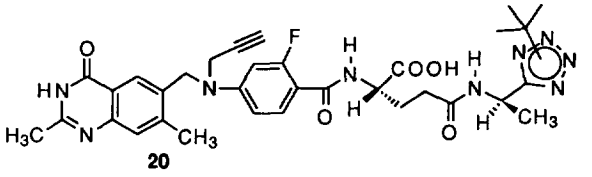
Scheme 1



Conditions: (a) i) Et_3N (1 eq), $ClCO_2CH_2CH(CH_3)_2$ (1eq), THF, -15°C, 10 min, ii) NH_3, -15°C to RT, 1h (64%); (b) pyridine (9 eq), *p*-toluenesulphonyl chloride (1.3 eq), CH_2Cl_2, 0°C to RT, 3h (78%); (c) NH_4Cl (1.1 eq), NaN_3 (1.1 eq), DMF, 90°C, 19h (70%); (d) H_2, 10% Pd/C, ethanol, 18h (98%); (e) i) N-methylmorpholine (1 eq), $ClCO_2CH_2CH(CH_3)_2$ (1eq), THF, -20°C, 10 min, ii) **14**, (1.05 eq), THF, -20°C to RT, 3.5h (86%); (f) H_2, 10% Pd/C, ethanol, 14h (89%); (g) PyBOP® (1.05 eq), DIEA (3 eq), DMF, 0°C to RT, 1.5h (44%); (h) TFA/H_2O 4.75h (88%).

Z-Blocked dipeptide derivative **16** was obtained in 86% yield by condensing **14** with α-*tert*-butyl N-benzyloxycarbonyl-L-glutamate (**15**) via isobutyl mixed anhydride coupling and followed by removal of the Z-group by catalytic hydrogenolysis using 10% palladium on charcoal. Initial condensation of **17** with the

pteroic acid analogue **18**,[10] employing diethylphosphorocyanidate (DEPC)[15] as coupling reagent, gave the ester **19** in only 22% yield after column chromatography and contaminated with an unidentified impurity. Pure product however was obtained, in higher yield (44%, after column chromatography), via benzotriazoloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate (PyBOP®) activation.[16] To our knowledge this is the first time that PyBOP® has been used for the condensation of amino acids or peptides with pteroic acid analogues. In this system PyBOP® was superior to DEPC. In the last step, treatment of **19** with trifluoroacetic acid (TFA) gave a mixture of two products: the desired antifolate **7** and 10% of a by-product which is believed to be the *tert*-butylated tetrazole derivative **20**.



20

We believe that **20** is formed by electrophilic substitution at either N-1 or N-2 of the tetrazole ring by the *tert*-butyl cation produced *in situ* during the acid catalysed hydrolysis of the antifolate ester **19**. Performing the TFA hydrolysis in the presence of water significantly suppressed the formation of **20** and the best results were obtained when a solution of **19** (0.15 mmol) in water (3.1 mL) and TFA (7.5 mL) was stirred at room temperature for 3.5 h, followed by the addition of more TFA (7.5 mL) and further stirring at room temperature for 1.25 h. Under these conditions formation of **20** was suppressed to 0.6% as estimated by HPLC (C18 reverse phase column, $\lambda = 230$ nm, flow rate = 2 mL/min, isocratic mobile phase: 60% MeOH / 40% H_2O containing 1% AcOH). The methodology developed for the preparation of **7** was also employed in the preparation of **8** and **9** using the appropriate pteroic acid analogue and α-*tert*-butyl N-benzyloxycarbonyl-D-glutamate (Z-D-Glu-OBu^t) in place of Z-D-Ala as the chiral building block. Z-D-Glu-OBu^t was prepared in 4 steps from D-glutamic acid through γ-allyl ester protection.[17]

Table 1: Inhibitory activities against TS, and L1210, L1210:1565 cell growth[a]

Compound	TS, IC_{50} (nM)	L1210, IC_{50} (μM)	L1210:1565, IC_{50} (μM)
3 (from ref 10)	4.6	0.33 ± 0.25	15, 16
5 (from ref 10)	0.4, 0.92	0.20 ± 0.017	6.8, 8.7
8	6.0, 4.0	6.2, 4.9	44, 41
9	0.82, 1.86	1.5, 0.75	8.0, 7.4
7	1.0	0.35, 0.26	2.0, 1.0

[a]Methodologies for the inhibition of mouse L1210 TS and L1210, L1210:1565 (impaired RFC) cell growth are as described in refs 18, 19.

Introduction of a tetrazole as a γ-carboxylate replacement in the ICI 198583-γ-D-Glu (**3**) gave the acid mimic **8** whose inhibitory activity against TS was similar to that of the parent compound (**3**) but with a markedly (18-fold) reduced inhibition of L1210 cell growth. The reasons for this poor cell growth inhibitory potency are not clearly understood although poor cellular uptake via the reduced folate carrier (RFC) may be a contributory factor. Compounds **3** and **5** are believed to use the RFC for cell entry as evidenced by their poor activity against the L1210:1565 cell line (impaired RFC). Although **8** and **9** also have poorer activity against this cell line compared with the L1210, the L1210:1565/L1210 ratio is lower than that of **3** and **5** respectively. A similarly low L1210:1565/L1210 ratio has also been observed for the AlaT derivative **7**. These results indicate the use of the RFC, but the significance of the low ratios may possibly suggest poorer cellular uptake of these tetrazole acid mimics via the RFC mechanism.

In conclusion, the acid mimics prepared in this study are potent inhibitors of TS and are comparable with the most potent γ-linked L, D dipeptide derivatives of ICI 198583 previously synthesised.[10] However, this potency did not lead to an enhancement in cell growth inhibition, possibly because other factors e.g. cell membrane transport, are compromised.

Acknowledgements

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