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Regioselective synthesis of peptidic derivatives and glycolamidic esters of Methotrexate

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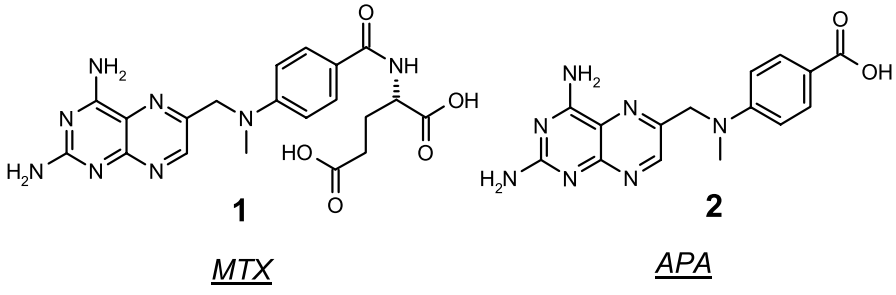
Abstract—Convenient methods for regioselective syntheses of Methotrexate peptidic conjugates are described. Solid phase synthesis for derivatives of Methotrexate containing an amide bond has been applied and showed higher efficiency than liquid phase synthesis. Synthetic pathways for regioselective preparation of Glycolamidic ester derivatives of Methotrexate were also developed using 4-amino-4-deoxy-N^{10}-methylpteroic acid as starting material. These ester bonds were obtained either in solution or by using a combined liquid and solid phase synthesis.

1. Introduction

Methotrexate (MTX) (Scheme 1), a folic acid antagonist, has been extensively used in cancer chemotherapy since 1948.[1] This drug acts as an inhibitor of dihydrofolate reductase (DHFR), an essential enzyme in the biosynthesis of thymidylate, which is required for DNA replication.[2] MTX is also subsequently used in the treatment of non-neoplasmic diseases as an anti-inflammatory and/or an immunosuppressive drug.[3] The mechanism of action of MTX is well documented. It is brought into the cell by the reduced folate transporter, and it is then polyglutamylated in order to prevent efflux from the cell and to exert its cytotoxic activity by blocking the synthesis of the N5, N10-methylenetetrahydrofolate.[3]

The efficacy of MTX is hampered by its very short plasma half-life. It is administrated in relatively high doses, which often leads to drug resistance and causes non-specific toxicities in normal cells.[1] Resistance to MTX is the result of different biological phenomena in the target cell. Deficiency in the reduced folate transporter, in folyl polyglutamate synthetase (FPGS) or over-expression of gamma-glutamyl hydrolase (GGS) and efflux proteins at the surface of the cell (MRP proteins), can prevent MTX to enter into and/or stay inside the cell. In the same time, over-expression and/or mutation of DHFR can prevent MTX to exert a sufficient cytotoxic activity.[4–6] Another important side effect of MTX which has been clearly identified, is its huge chronic toxicity for the central nervous system when the drug is used concomitantly with radiations.[7]



Scheme 1. Structures of MTX and APA.

Keywords: Methotrexate; Peptidic conjugate; Regioselective synthesis; Solid phase synthesis; Vectorisation.
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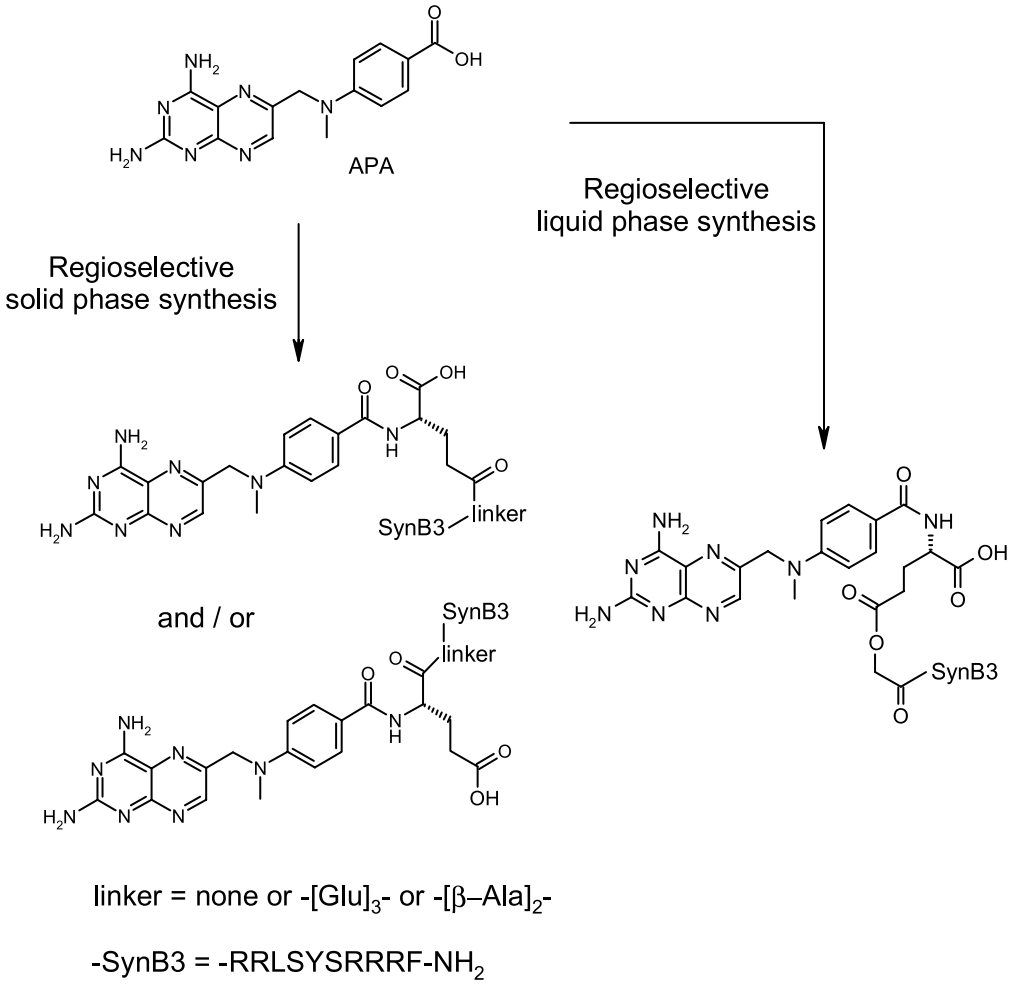
To overcome these problems, many experimental efforts have been made. Among them, modifications of MTX are the most employed. γ-*tert*-Butyl ester, γ-hydrazide, γ-*n*-butylamide and γ-benzylamide of MTX were synthesized in order to modify the biological behaviour of the drug.[8] Other compounds with amino acids and various esters on the two carboxylic acid functions were also prepared with different strategies, the most current one involving the use of 4-amino-4-deoxy-N^{10}-methylpteroic acid (APA) (Scheme 1) as a building block.[1,9–11] These transformations were performed in order to modify the lipophilicity of MTX and/or to study the cytotoxicity of the prodrug activated by various enzymes. In all cases, either the regioselective synthesis described involve numerous steps, or the final products are obtained after purification of the formed isomers.

Instead of modifying the core structure of MTX, vectorisation is an alternative strategy to increase the efficacy of MTX. MTX has been attached to various vectors like monoclonal antibodies,[12,13] proteins,[14] polymers[15,16] and hormonal peptides.[17–19] All the synthesis are performed in liquid phase with or without protecting groups, leading, respectively, to a single product with a relatively low yield, or to a mixture of regioisomers. More recently, Pignatello and co-workers have described the synthesis of several α- and γ-monosubstituted and α,γ-disubstituted lipoamino acid conjugates of MTX coupled with a glycolamidic ester.[20] Once again, the monosubstituted products are obtained as secondary products of the disubstituted ones, and the yields do not exceed 15% for these compounds.

Previous work in our laboratory led to the discovery of peptides derived from the Protegrin PG-1 peptide, which belongs to the family of β-stranded antimicrobial peptides.[21] These peptides have been shown to translocate efficiently through biological membranes, thus providing the basis for the development of new peptide-conjugated drugs that cross-cell membranes. In a precedent work,[22] the authors have shown that vectorized doxorubicin bypasses the P-glycoprotein pump at the luminal site of the blood-brain barrier. Moreover, using these peptidic vectors with doxorubicin is efficient in overcoming multidrug resistance. In the present paper, we report the synthesis of MTX peptidic conjugates using solid phase synthesis for the conjugate with amide bonds between the vector and the drug. A regioselective liquid phase synthesis will also be presented for conjugates containing a glycolamidic ester bond (Scheme 2).



Scheme 2. Target molecules and general strategies used.