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The synthesis of possible transition state analogue inhibitors of thymidine phosphorylase

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ABSTRACT

The synthetically challenging S_N2 transition state mimic for thymidine phosphorylase, along with its phosphonate analogue, were synthesised via a modified Corey–Link reaction in good overall yields and ensuring the correct stereochemical outcome.

Enzymes comprise one of the major categories of drug targets pursued by the pharmaceutical and biotechnology industries.[1] Historically, small molecule inhibitors of enzyme function have afforded a significant proportion of FDA approved drugs.[2] Enzymes have been optimised by Nature to make and break covalent chemical bonds as opposed to ligand binding.[1] The methodology used to design enzyme inhibitors has progressed markedly over the past quarter of a century and shares many aspects of ligand design in general.[3] These design aspects can include knowledge of the natural ligand or substrate of the target of interest or of another compound already known to interact with the target of interest, screening of both random and biased libraries against a target, fragment-based screening (which can involve a variety of physical methods of analysis but mostly centre around X-ray crystallography and NMR).[3a]

As an alternative to these ground state methods of inhibitor design, transition state affinity[4] of enzymes and the design of transition state analogs should be considered as the preeminent method by which to afford both potent and selective inhibitors of enzymes.[1,3a,5] Pauling first posited that enzymes had evolved or were designed to recognise the activated state of reactants. This premise was restated by Leinhard[5b] and Wolfenden[4] that the transition state binds more tightly than the substrate(s) and so accounts for catalysis. Schramm[6] has developed methods that can "image" the activated state of reactants or the transition state, on-enzyme, and we can then develop a blueprint for the design of selective tight binding inhibitors. Some of these inhibitor designs have been realised over the past two decades[7] and have been shown to possess the advantages of covalently bound inhibitors without the off-site activity that sometimes impacts on the utility of this class of enzyme inhibitor.[8]

Thymidine phosphorylase (TP) is an enzyme that catalyses the reversible phosphorolysis of thymidine into thymine and 2′-deoxy-D-ribose 1-phosphate. TP is an enzyme important in the salvage of pyrimidine nucleosides, and to promote angiogenesis.[9] Improved vasculature is necessary for the growth of solid tumours and the expression of this enzyme in many solid tumours correlates with the aggressiveness and invasiveness of the cancer.[9c,f,10] TP inhibitors affect the production of 2′-deoxy-D-ribose, and in turn suppress tumour growth. Consequently, inhibitors of TP are expected to be useful as anti-cancer compounds.[11]

Schramm's initial design blueprint for a transition state analogue inhibitor of human TP, was derived using an enzyme construct with purification tags, and suggested an S_N2-like transition state, with a bond order of 0.5 to the leaving group and 0.33 to the attacking oxygen nucleophile of the phosphate, which was unprecedented for *N*-ribosyl transferase. This transition state supports continuous electron density extending from N1 through C1 to the phosphate oxygen as expected for an S_N2 transition state, that is, with equal participation of both the incoming phosphate

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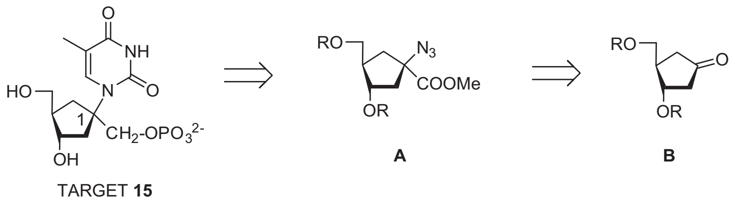


Figure 1. Retrosynthetic analysis of the transition state analogue target.

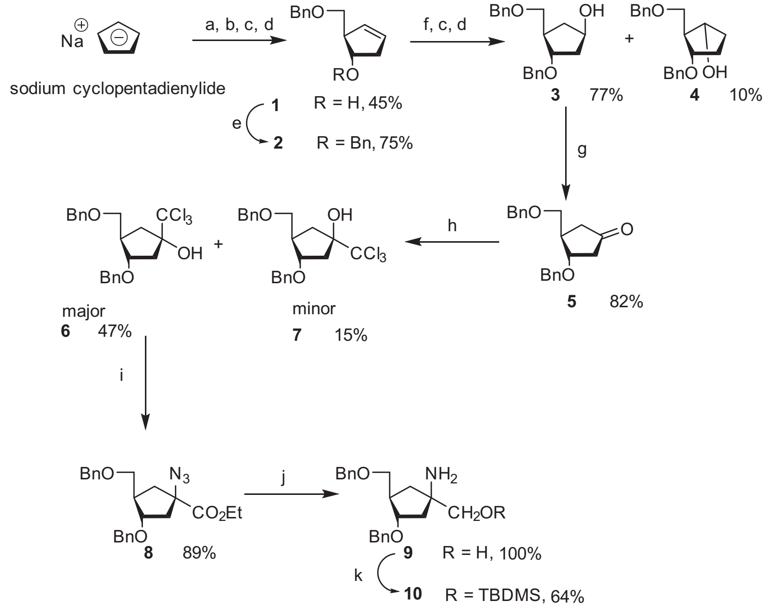
nucleophile and pyrimidine leaving group with little or no charge at the reaction centre of the ribosyl moiety.[12] With this background, we embarked on the synthesis of analogs geometrically and electronically similar to the S_N2 transition state. The novel chemistry of their synthesis is described here. They were not significant inhibitors of the enzyme, supporting a different transition state for TP.

Subsequent hydrolytic[13] and arsenolytic[6d] transition state analysis with native human TP, and without the protein-modifying purification tags, supported a transition state with ribocationic character with little or no participation of the incoming nucleophile in an A_ND_N mechanism.

Regardless of these latter results, incorporating both a thymine or thymine mimic and a phosphate or phosphate mimic at a single carbon of a cyclopentane ring, with a methylene group between the phosphate and the ring as a 'spacer' provided a real synthetic challenge (Fig. 1). The resulting compounds proved useful in eliminating the earlier S_N2 transition state proposal for TP. The present chemistry provides a useful precedent for the synthesis of similar compounds for related enzyme chemistry.

Ludek and Meier[14] previously described the synthesis of a carbocyclic thymidine analogue, and so we investigated ways in which this procedure could be modified to afford our desired target compound. The core cyclopentane structure could be realised via the alkene intermediate **1**, which utilised an enantioselective hydroboration of cyclopentadiene first described by Biggadike et al.,[15] and inspired by Partridge et al. (Scheme 1).[16] The physical characteristics of alkene **1** were the same as those reported in the literature and it was benzylated to yield compound **2** and then hydroborated using borane to afford a mixture of alcohols **3** and **4**, which could be separated by chromatography.[17] Oxidation of the alcohol **3** using PDC afforded the key ketone intermediate, compound **5**,[17] in good overall yield from cyclopentadiene.

Consideration was now given to the stereochemical outcome of methods appropriate for installing the nitrogen and carbon functionalities C1′, where the nitrogen required to construct the thymine moiety is above the ring plane and the carbon, which will provide the methylene 'spacer' between the ring and the phosphate moiety, is below. We were influenced by the stereochemical analysis of Stick and co-workers on a carbohydrate ketone in their study and the likely outcomes of a Strecker reaction[18] versus that of a modified Corey–Link reaction.[19] Corey and Link first described the synthesis of α-amino acids via the reaction of a trichloromethylcarbinol with NaOH and NaN_3 in 1992.[20] This was followed by a modification of this procedure by Domínguez et al., exemplified in a ring system not dissimilar to our own, using DBU and MeOH to afford the desired α-azido methyl ester.[21] Further literature precedent[21,22] demonstrated the utility of the modified Corey–Link reaction and so we were keen to adopt this approach in the setting described above. We considered that the addition of a sterically, and hence, stereochemically demanding trichloromethylcarbanion would occur predominantly from the least hindered β face of the cyclopentanone ring and therefore, where the Corey–Link reaction with azide, base and the intermediate *gem*-dichlorooxirane



Scheme 1. Reagents and conditions: (a) BOMCl, THF –60 °C → –20 °C; (b) (–)-diisopinan-3-yl borane, THF, –60 °C → –20 °C; (c) 3 M NaOH, 0 °C; (d) 30% H_2O_2, THF, 0 °C → rt; (e) BnBr, NaH, DMF, 0 °C → rt; (f) 9-BBN, THF, 0 °C → rt; (g) PDC, Ac_2O, CH_2Cl_2, rt; (h) $CHCl_3$, LHMDS, THF, –78 °C → rt; (i) NaN_3, DBU, EtOH, 50 °C; (j) LAH, THF, 0 °C → rt; (k) TBDMSCl, imidazole, DMF, rt.