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Solid supported synthesis of oligonucleotide conjugates of the antitumour drug temozolomide

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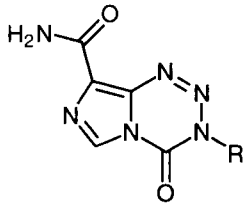
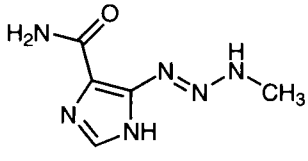
With its clinical benefits against brain tumours and malignant melanoma, the antitumour drug temozolomide (**1**) continues to attract sustained interest (Wang et al 1997). The drug (**1**) is thought to interact with DNA by binding to the major groove where, on hydrolytic ring opening and decarboxylation to MTIC (**3**), causes alkylation of guanine, predominantly at O6 (Clark et al 1995). With the aim of achieving sequence-specific targeting of **1** to the major grove of DNA, we investigated the possibility of synthesising triplex-forming oligonucleotide (TFO) conjugates of **1** and, additionally, the DNA cross-linking agent mitozolomide (**2**) (Walsh et al 1996). Synthesis of TFO conjugates has traditionally relied on post-synthetic attachment of the auxiliary molecule using solution phase chemistry which is time consuming, indirect, low yielding and can involve several purification stages. Our aim was to develop a direct and general method for solid supported synthesis of TFO conjugates containing **1** and **2**. Although robust under acidic conditions, **1** is particularly sensitive to base, being rapidly hydrolysed above pH 7. This property renders **1** incompatible with the standard supports and deprotection conditions used for conventional TFOs. We recently reported an improved synthesis of our novel Silyl-Linked Controlled Pore Glass (SLCPG) solid support (Walsh et al 1997) which we developed specifically for synthesis of base-labile oligomers and their conjugates. SLCPG can be cleaved at neutral pH using fluoride (Routledge et al 1995). During the course of the present work we demonstrated efficient hydrolytic cleavage of the support under mild acidic conditions; our preferred method, compatible with TFO conjugates of **1** and **2**.



1 R = CH_3 temozolomide
2 R = CH_2CH_2Cl mitozolomide



3 MTIC

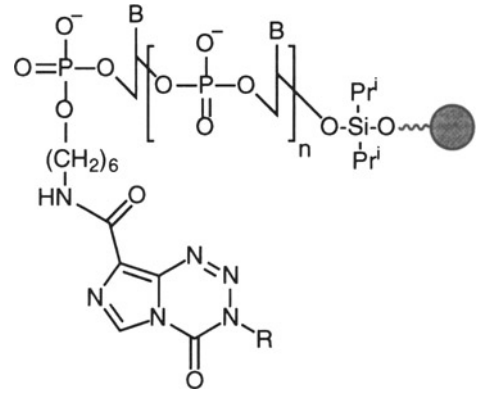


Figure. Conjugate Synthesis on SLCPG

TFOs were synthesised on SLCPG using phosphoramidite chemistry. An amino-hexyl linker was installed at the 5'-end for attaching to the drugs. Amino protecting groups (*t*-BPA) and the cyanoethyl groups at phosphorous were removed using firstly ethanolamine, followed by *tert*-butylamine, to provide fully deprotected, solid-supported TFOs which were subsequently reacted with an excess of the carboxylic acid derivatives of **1** and **2** to form conjugates (Figure). Conjugates were released from the support on mild acid hydrolysis of the silyl anchor. Small molecular weight components were removed by size-exclusion chromatography and the crude conjugates analysed and purified to homogeneity by reversed-phase HPLC. Characterisation of conjugates of **1** was achieved using electrospray mass spectrometry with molecular ions calculated from the mass-to-charge ratios of the intact, multiply charged sodium adducts. TFO conjugates of **2** were also successfully prepared although electrospray mass analysis of the purified materials indicated ionisation-induced decomposition of the tetrazinone ring during the mass analysis.

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