Towards Selective DNA Targeting: Synthesis of an Antibody-Macrocycle-Intercalator Conjugate

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The synthesis is reported of a trivalent conjugate incorporating a macrocyclic bifunctional complexing agent, an engineered human monoclonal antibody fragment and an established DNA intercalator.

In radioimmunotherapy, the target is the chromosomal DNA of tumour stem cells. Given that an antibody can be found to bind selectively to an antigen found uniquely on the tumour cell surface, and if a fairly long-range $\beta^-$-emitting radioisotope (e.g. $^{90}$Y, $t_1$ 64 h, mean range in tissue 3.9 mm) is conjugated to the protein, then the overall cytotoxic effect is mainly due to 'crossfire' from neighbouring cells. The monoclonal antibody therefore affords a means of selective cellular localisation. With the advent of 'internalisable' antibodies that may enter the cytoplasm following mediated endocytosis, the possibility of targeting a suitable modified antibody to the DNA, at the molecular level, becomes plausible. At the S stage of the cell cycle, when the cellular DNA is synthesised and is most likely to be radiosensitive, a DNA-binding conjugate could in principle seek out the molecular target. In theory, localisation at this molecular level could allow the use of shorter-range therapeutic isotopes as an adjunct to therapy with more energetic longer range particles. Examples of the former class include the Auger-emitting $^{111}$In$^+$ (Auger mean energy, 0.5 to 25 keV, range in tissue 20 nm to 8.5 $\mu$m), $^{123}$I, $^{99m}$Tc and $^{64}$Cu. Copper-64 emits Auger electrons with energy in the range 6.5 to 0.8 keV (range 1.3 $\mu$m to 38 nm) but it is also a $\beta^-$ (mean $E = 190$ keV) and positron-emitting isotope (mean $E = 278$ keV, $t_1$ 12.8 h). It could provide a dual therapeutic effect: longer range killing (crossfire to adjacent cells) from the $\beta^-$ particles, coupled with the short-range killing of the Auger electrons. Simultaneous imaging of the positrons using PET (positron emission tomography) adds to the appeal of $^{64}$Cu-based therapy.

With these thoughts in mind we have prepared, in a modular manner, conjugates consisting of an antibody linked to both a bifunctional complexing agent, for radioisotope labelling, and to a known DNA intercalator. Acridines substituted in the 9-position are known to bind to the major-groove of DNA, and have been used in these exploratory studies. Reaction of the bifunctional complexing agent, with the $N$-protected $\alpha$-chloroacetylbutanamide (DMF, K$_2$CO$_3$) afforded the carbamate in 65% yield. Deprotection with wet TFA gave the primary amine which was reacted with acridine-9-carboxylic acid chloride (CH$_2$Cl$_2$, Et$_3$N) to yield the amide in 50% yield after purification by reverse-phase HPLC. Deprotection of the p-methoxybenzenesulfonyl group (HBr, AcOH, PhOH, 100 $^\circ$C, 15 h) led to the amine (72%) and subsequent reaction with 4-maleimido butyryl chloride under forcing conditions (CH$_2$Cl$_2$, DMF, Et$_3$N, 20 $^\circ$C) gave the desired maleimide, as a 2:1 mixture of diastereoisomers, in 93% yield after HPLC purification. Variable temperature $^1$H NMR studies (5–40 $^\circ$C) suggested that the two isomers were amide rotamers with a relatively high ($\Delta G^\circ > 70$ kJ mol$^{-1}$) barrier to rotation about the NCO bond because of intramolecular hydrogen bonding. Incubation of 7 with a humanised A-33 A-Cys-Fab fragment gave the conjugate which was purified by gel filtration HPLC. For purposes of comparison, the macrocycle conjugates 11 and 10, without the intercalator and without the protein respectively, were also prepared using similar methodology.

![Chemical structures](image-url)
undertaken. Reaction of with oxalyl chloride gave the acid chloride incubated with yield (8%) after HPLC purification. This trimaleimide was with succinic anhydride, (Et3N, DMF) followed by treatment with tri-Fab conjugate, as deduced by gel-filtration HPLC. Given that a tri-Fab binds the antigen more avidly than a mono-Fab and internalises into cells more readily,10 the synthesis of a tri-Fab-macrocycle-acridine conjugate, 13, was undertaken. Reaction of 12a (see preceding communication) with succinic anhydride, (Et3N, DMF) followed by treatment with the amine, (Et,N, DMF) afforded the trimaleimide, 13, albeit in poor yield (8%) after HPLC purification. This trimaleimide was incubated with 5 equiv. of h.A-33 Fab, to generate the desired tri-Fab conjugate, as deduced by gel-filtration HPLC.

Alternative strategies exist for the synthesis of conjugates incorporating an antibody-macrocycle-intercalator. If the intercalator merely needs to be non-specifically linked to an antibody-bifunctional complexing agent conjugate (e.g. via acylation of Lys ε-amino groups), then the active ester, 14, is useful for this purpose. Reaction of a 5-fold excess of N3-methyldihylenetriamine with acridine-9-carbonyl chloride followed by reaction with bis-(p-nitrophenyl)succinate (in DMSO) gave the active ester, 14, in a 61% overall yield after reverse-phase HPLC purification. The presence of the easily protonated N-methyl group will be expected to enhance the DNA-binding ability of derived conjugates.11,12 The results of cell-killing experiments, initially with labelled conjugates, e.g. 8, (with labelled 10 and 11 as controls), should allow the efficacy of these systems to be evaluated. We thank the NECRC and EPSRC for support.

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Footnotes
† Indium-111 (as the oxine complex) has been shown to be capable of inducing cell-death,4 and has an energy deposition of 19 eV nm−1 with a range in tissue of 1.3 μm to 1.5 nm. It is also a useful imaging isotope for immunoscintigraphy (γ, t, 2.83 d), and binds strongly to 9-N3 and 12-N3 based macrocyclic ligands.6 The criterion for a single strand break (often reparable) in DNA is an energy deposition of about 20 eV strand−1 or 10 eV nm−1, and for a double strand break (usually irreparable) is that the lesions in opposite strands should be within 16 nucleotide pairs of each other.14
‡ Compounds gave [H, 31P NMR and mass spectra in accord with the proposed structures and were judged homogeneous by reverse-phase analytical HPLC: e.g. 7: bH (pD 4) 29.1, 24.8 (1:2); bH (pD 4) 1.42 (6 H, d, d, J = 14 Hz), 1.48 (3 H, d, PMe), 1.32-1.62 [4 H, br m, (CH2)2(CH2-NCO)], 1.65-1.78 (4 H, m (CH2)2(CH2-NHCO-Ar)), 1.82 (2 H, quint, NCH2CH2CH2-CO), 2.28 + 2.45 (2 H, n, N(CH2)2CH2-CO), 3.05-3.60 [30 H, m, CH3-N ring + NHCH2P + NHCH2CH2-CO] + NHCH2CH2CH2NCO + CH2-NHCOCH2-NCO] 3.72 (2 H, s, CH2-NHCO-Ar), 3.78 [2 H, s, (CH3)2NCH2-CO], 3.98 + 4.13 [2 H, s, s (2:1), CONCH2-CO], 6.76 + 6.78 (2 H, s, s, CH=CH, 2:1) 7.99 (2 H, I, H6 + H8), 8.25 + 8.40 (d + t + d; 6 H, H5, H7, H6, H8, H9, H10). ms (ESMS) 1075 (M+ + 1).}

References
9 D. J. King and G. T. Yarranton, unpublished results.