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

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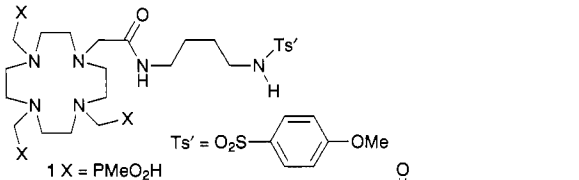
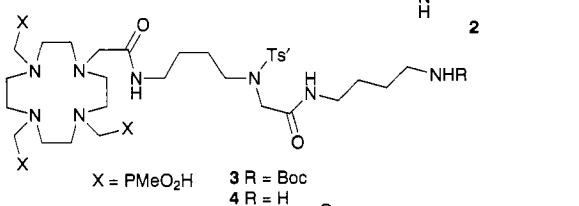
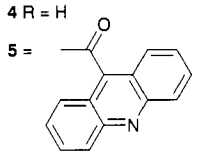
The synthesis is reported of a trivalent conjugate incorporating a macrocyclic bifunctional complexing agent, an engineered human monoclonal antibody fragment and an established DNA intercalator.

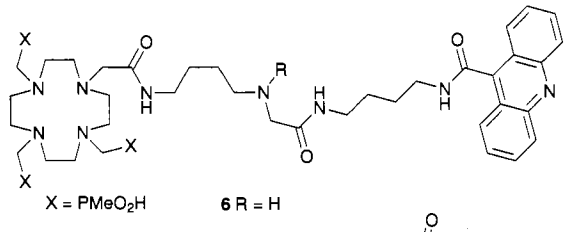
In radioimmunotherapy, the target is the chromosomal DNA of tumour stem cells.[1] Given that an antibody can be found to bind selectively to an antigen found uniquely on the tumour cell surface, and if a fairly long-range β⁻-emitting radioisotope (*e.g.* ^{90}Y, $t_{\frac{1}{2}}$ 64 h, mean range in tissue 3.9 mm) is conjugated to the protein, then the overall cytotoxic effect is mainly due to 'crossfire' from neighbouring cells.[2] The monoclonal antibody therefore affords a means of selective cellular localisation. With the advent of 'internalisable' antibodies[3] 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[4,5] radioisotopes $^{111}In^{\dagger}$ (Auger mean energy, 0.5 → 25 keV, range in tissue 20 nm → 8.5 μm), ^{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 μm to 38 nm) but it is also a β⁻ (mean E = 190 keV) and positron-emitting isotope (mean E = 278 keV, $t_{\frac{1}{2}}$ 12.8 h). It could provide a dual therapeutic effect: longer range killing (crossfire to adjacent cells) from the β⁻ 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.

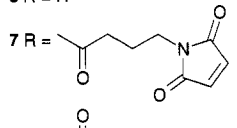
With these thoughts in mind we have prepared, in a modular manner, conjugates consisting of an antibody linked to both a bifunctional complexing agent, for radioisotope labelling,[6] and to a known DNA intercalator. Acridines substituted in the 9-position are known to bind to the major-groove of DNA,[7] and have been used in these exploratory studies. Reaction of the bifunctional complexing agent, **1**,[8] with the *N*-protected α-chloroacetylbutanamide **2** (DMF, K_2CO_3) afforded the carbamate **3** in 65% yield. Deprotection with wet TFA gave the primary amine **4** which was reacted with acridine-9-carboxylic acid chloride[13] (CH_2Cl_2, Et_3N) to yield the amide **5** in 50% yield after purification by reverse-phase HPLC. Deprotection of the *p*-methoxybenzenesulfonyl group (HBr, AcOH, PhOH, 100 °C, 15 h) led to the amine **6** (72%) and subsequent reaction with 4-maleimido butyryl chloride under forcing conditions (CH_2Cl_2, DMF, Et_3N, 20 °C) gave the desired maleimide, **7**,‡ as a 2:1 mixture of diastereoisomers, in 93% yield after HPLC purification. Variable temperature ^1H NMR studies (5–40 °C) suggested that the two isomers were amide rotamers with a relatively high ($\Delta G^{\ddagger}$ > 70 kJ mol^{-1}) barrier to rotation about the NCO bond (**9**) because of intramolecular hydrogen bonding. Incubation of **7** with a humanised A-33 Δ-Cys-Fab fragment[9] gave the conjugate **8** 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.§

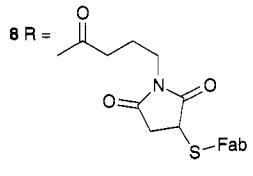


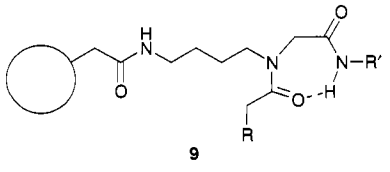


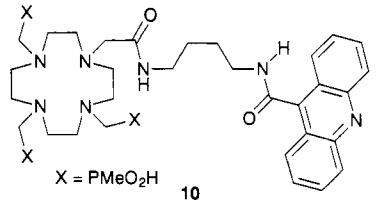


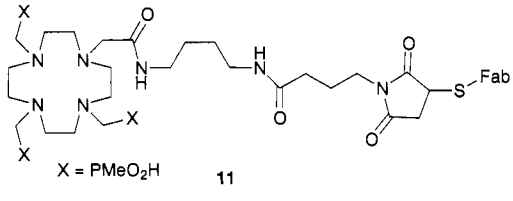


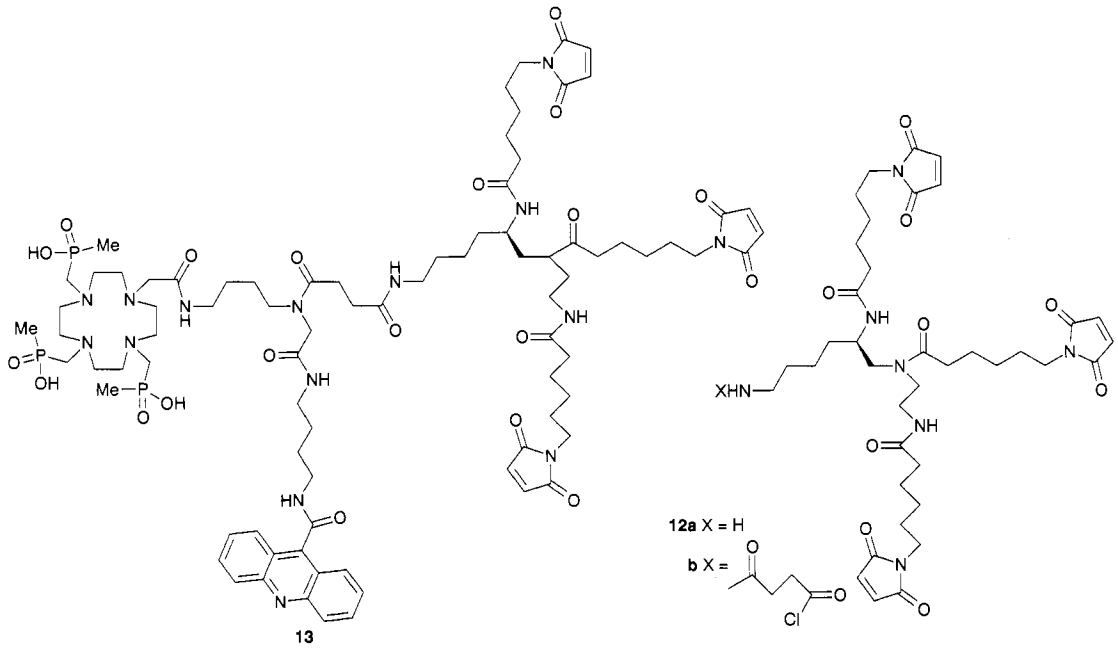












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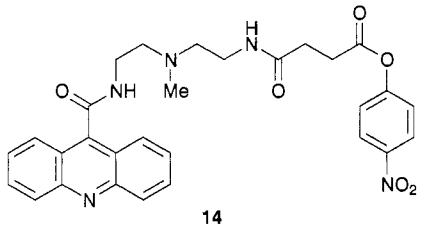

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Given that a tri-Fab binds the antigen more avidly than a mono-Fab and internalises into cells more readily,[10] the synthesis of a tri-Fab-macrocycle-acridine conjugate, **13**, was undertaken. Reaction of **12a** (see preceding communication) with succinic anhydride, (Et_3N, DMF) followed by treatment with oxalyl chloride gave the acid chloride **12b** (ν_{CO} = 1770 cm^{-1}) almost quantitatively. Subsequent addition of the amine, **6**, (Et_3N, 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 N^2-methyldiethylenetriamine 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 ^{111}In labelled conjugates, *e.g.* **8**, (with labelled **10** and **11** as controls), should allow the efficacy of these systems to be evaluated.

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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, $t_{\frac{1}{2}}$ 2.83 d), and binds strongly to 9-N_3 and 12-N_4 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 ^{1}H, ^{31}P NMR and mass spectra in accord with the proposed structures and were judged homogeneous by reverse-phase analytical HPLC: *e.g.* **7**: δ_P (pD 4) 29.1, 24.8 (1:2); δ_H (pD 4) 1.42 (6 H, d, PMe, J = 14 Hz), 1.48 (3 H, d, PMe), 1.32–1.62 [4 H, br m, $(CH_2)_2CH_2NCO$], 1.65–1.78 (4 H, m $(CH_2)_2CH_2NHCO$-Ar), 1.82 (2 H, quint, $NCH_2CH_2CH_2CO$), 2.28 + 2.45 (2 H, tt, $N(CH_2)_2CH_2CO$), 3.05–3.60 [30 H, m, CH_2N ring + NCH_2P + $NCH_2(CH_2)_2CO$] + $NHCH_2(CH_2)_2CH_2NCO$ + $CH_2NHCOCH_2NCO$) 3.72 (2 H, t CH_2NHCO-Ar'), 3.78 [2 H, s, $(CH_2)_2NCH_2CO$], 3.98 + 4.13 [2 H, s + s (2:1), $CONCH_2CO$], 6.76 + 6.78 (2 H, s + s, CH=CH, 2:1) 7.99 (2 H, t, H_6 + H_3), 8.25 + 8.40 (d + t + d; 6 H, H_5, H_4, H_7, H_2, H_8, H_1). *m/z* (ESMS) 1075 (M^+ + 1).

§ Using intermediates and methods defined in the preceeding communication.

References

1 D. Pressman, *Ann. N.Y. Acad. Sci.*, 1957, **69**, 644.
2 J. Humm, *J. Nucl. Med.*, 1986, **27**, 1490.
3 S. Welt, C. R. Divgi, F. X. Real, S. D. Yeh, P. Garin-Chesa, C. L. Firstad, J. Sakamoto, A. Cohen, E. R. Sigurdson, N. Kememy, E. A. Carswell, H. F. Oettgen and L. J. Old, *J. Clin. Oncol.*, 1990, **8**, 1894.
4 ICRP Publication 38, *Radionuclide Transformations*, Pergamon Press, 1983; A Birchall, *Health Physics*, 1986, **50**, 389.
5 D. A. Lea, *Action of Radiations on Living Cells*, CUP, 1956.
6 D. Parker, *Chem. Soc. Rev.*, 1991, **19**, 271.
7 L. P. G. Wakelin and M. J. Waring, *DNA Intercalating Agents* in *Comprehensive Medicinal Chemistry*, vol. 2, Pergamon, Oxford, 1989; C. Norbury and P. Nurse, *Ann. Rev. Biochem.*, 1992, **61**, 441.
8 K. P. Pulukkody, T. J. Norman, D. Parker, L. Royle and C. J. Broan, *J. Chem. Soc., Perkin Trans. 2*, 1993, 605.
9 D. J. King and G. T. Yarranton, unpublished results.
10 M. E. Schott, K. A. Frazier, D. K. Pollock and K. M. Verbanac, *Bioconjugate Chem.*, 1993, **4**, 153; D. J. King, A. Turner, A. P. H. Farnsworth, J. R. Adair, R. J. Owens, R. B. Pedley, D. Baldock, K. A. Proudfoot, A. D. G. Lawson, N. R. A. Beeley, K. Millar, A. T. Millican, B. A. Boyce, P. Antoniw, A. Mountain, R. H. J. Begent, D. Schochat and G. T. Yarranton, *Cancer Res.*, 1994, **54**, 6176.
11 U. Pindur, M. Haber and K. Sattler, *J. Chem. Ed.*, 1993, **70**, 263; E. C. Long and J. K. Barton, *Acc. Chem. Res.*, 1990, **23**, 271.
12 K. Shinozuka, Y. Seto and H. Sawi, *J. Chem. Soc., Chem. Commun.*, 1994, 1377.
13 S. Takenaka, S. Nishira, K. Tahara, H. Kondo and M. Takagi, *Supramolecular Chem.*, 1993, **2**, 41.
14 D. Charlton and J. Humm, *Int. J. Radiat. Biol.*, 1988, **53**, 353.