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4-Thio-5-bromo-2′-deoxyuridine: chemical synthesis and therapeutic potential of UVA-induced DNA damage

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Abstract—4-Thio-5-bromo-2′-deoxyuridine (**3a**) is prepared from 5-bromo-2′-deoxyuridine (BrdU) and its key properties are explored. The thionucleoside (**3a**) can react readily with monobromobimane and produces high fluorescence. **3a** has UV maximum absorption at 340 nm and can be incorporated into cellular DNA. The cells containing **3a** become sensitive to UVA light, offering therapeutic potential for UVA-induced cell killing.

1. Introduction

DNA is frequently damaged by internal and external agents. Damaged DNA, if not repaired, can lead to mutation and cancer.[1] On the other hand, purposely designed DNA damage can be used as a means to kill cancer cells. This approach underlies chemotherapy and radiotherapy in which a toxic chemical or high energy radiation is employed to inflict lethal DNA damage on cancer cells. However, these treatments are undiscriminating and also harm normal cells. An ideal treatment would be to target DNA in cancer cells selectively while minimising damage to DNA in normal cells. Recently we reported[2,3] that 4-thiothymidine (S^4-TdR), an analogue of the naturally occurring nucleoside thymidine, can be incorporated into cellular DNA. More importantly, cells containing thiothymidine DNA are highly sensitive to low doses of UVA light.[3] These findings suggested a new therapeutic approach in which a minimally mutagenic, non-toxic pro-drug (e.g., S^4-TdR) is introduced into the DNA of target cells and then activated by a localised treatment with UVA light to execute cell killing. This mild and synergistic approach has the advantage over conventional therapy that it targets proliferating cells selectively. While investigating its killing mechanism, we also embarked on a search for novel or improved pro-drugs for UVA-induced cell killing.

To be suitable, a pro-drug of this kind should fulfil two basic requirements: (1) ready incorporation into cellular DNA, ideally with some selectivity towards cancer cells; (2) having UV maximum absorption substantially remote from 260 nm. As a part of our ongoing work, we have focused initially on thymidine analogues. Incorporation of thymidine analogues into cellular DNA requires their phosphorylation by the enzyme thymidine kinase (TK). TK activity is up-regulated in growing cells-including cancer cells. This can offer a level of selection against cancer cells. An additional level of targeting cancer cells would be achieved by selective UVA light activation and damage of the cells incorporating these thymidine analogues. Here we describe the first synthesis of 4-thio-5-bromo-2′-deoxyuridine (S^4-BrdU) **3a** and its key properties.

The synthesis of S^4-BrdU was carried out by a modification of the protocol we developed[4] for 4-thiothymidine. 5-Bromo-2′-deoxyuridine (BrdU) **1a** (Scheme 1) was reacted with trimethylchlorosilane to form 3′,5′-*O*-bis-(trimethylsilyl)-5-bromo-2′-deoxyuridine **1b**[5] which was then reacted with tri-triazolo oxyphosphorus produced in situ from triazole and $POCl_3$ to form 4-triazolo derivative **2b**.[7] **2b** was then transformed by a treatment with thioacetic acid into a 4-thio derivative **3b** which, after acidic deprotection, produced the title compound

Abbreviations: S^4-T, 4-thiothymine; S^4-TdR, 4-thiothymidine; dU, 2′-deoxyuridine; BrdU, 5-bromo-2′-deoxyuridine; S^4-BrdU, 4-thio-5-bromo-2′-deoxyuridine; TdR, thymidine; ELISA, enzyme linked immuno sorbent assay; TK, thymidine kinase; UVA, UV light at 320–400 nm; XP, Xeroderma Pigmentosum; mBB, monobromobimane.

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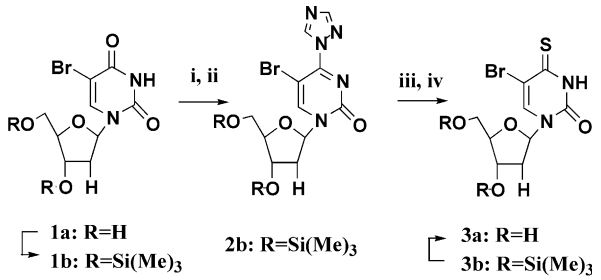


Scheme 1. Synthetic route to 4-thio-5-bromodeoxyuridine (S^4-BrdU). Reagents: (i) Cl–$Si(Me)_3$/TEA; (ii) Triazole/$POCl_3$/TEA; (iii) CH_3COSH; (iv) 1N HCl.

3a.[8] The overall yield from **1** to **3a** (4 steps) was 30%. To the best of our knowledge, this is the first report of the synthesis of 4-thio-5-bromo-2′-deoxyuridine. Interestingly, a great number of 5-substituted-2′-deoxyuridine analogues have been synthesised and tested for anti-viral or anti-cancer activity.[9] However, to our surprise, only very few 4-thio-5-substituted-2′-deoxyuridine analogues have been prepared[10] and little biological exploitation carried out.[11]

The current report of 4-thio-5-bromo-2′-deoxyuridine should provide a good model for chemical synthesis and biological evaluation of other 4-thio-5-substituted-2′-deoxyuridine analogues, particularly 4-thio-5-iodo-2′-deoxyuridine.

2. Properties of S^4-BrdU

2.1. Cellular incorporation

Initial experiments indicate that the synthetic S^4-BrdU can be incorporated into DNA by cultured human tumour cells. This incorporation is via TK activation to form its corresponding nucleotide and triphosphate, offering a potential for targeting TK-rich cells (such as tumour cells). Interestingly unlike S^4-TdR, S^4-BrdU in DNA can be recognised by the monoclonal antibodies used against DNA containing BrdU.[12] This recognition can be ascribed to the presence of a bromo group in S^4-BrdU and would endow S^4-BrdU with a unique property for its rapid detection in DNA by a simple ELISA procedure. Furthermore, like S^4-dT, S^4-BrdU was not toxic to cells over a range of concentrations from 0–300 μM. Several cell lines were tested and all of the cell lines[13] proliferated normally in the presence of up to 300 μM S^4-BrdU.

2.2. Photo absorption

The synthesised **3a** (S^4-BrdU) absorbs maximally at 340 nm (Fig. 1). This is substantially away from the absorption range (260–270 nm) of normal DNA nucleosides. As anticipated, the enhancement of conjugation leads to absorption by the nucleoside at longer wavelength (Table 1). Since the maximum wavelength for S^4-BrdU is further away, UVA activation of S^4-BrdU should inflict less damage to normal DNA and enhance the selectivity of cell-targeting.

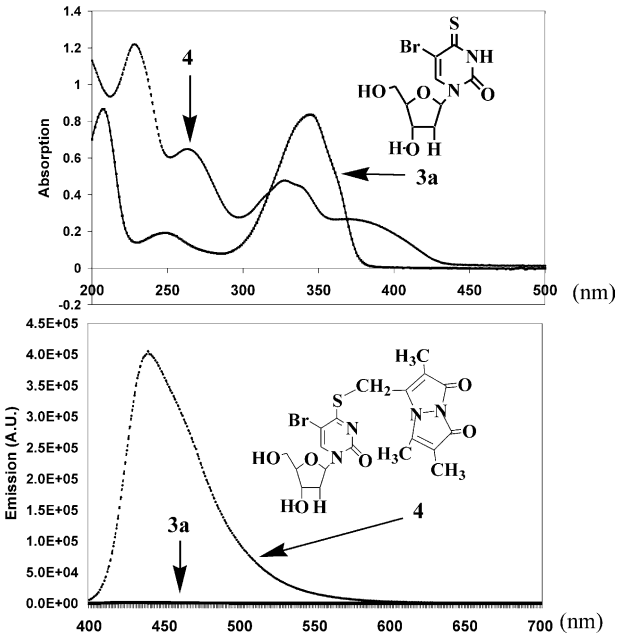
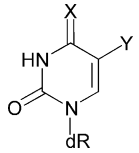


Figure 1. Top panel is the UV spectra of **3a** (50 mM in water) and **4** (50 mM in CH_3CN); Low panel is the fluorescence spectra of **3a** and **4** (excited at 380 nm). **3a** has little fluorescent emission.

Table 1. Chemical structures and maximum UV absorption of dU, TdR, BrdU and their thio-analogues



X	Y	λmax (nm)
O	H	262
O	CH_3	267
O	Br	280
S	H	332
S	CH_3	335
S	Br	340

2.3. UVA irradiation and its mechanisms

S^4-BrdU is not cytotoxic on its own but sensitises cells to killing by a subsequent exposure to low doses of UVA light. UVA irradiation (wavelength 320–400 nm, λmax 365 nm) has been used to cause lethal DNA damage selectively in cells that have incorporated S^4-BrdU into their DNA. Previously we suggested[3] that the extreme sensitivity of XP cells (Xeroderma Pigmentosum cells defective in nucleotide excision repair enzymes) to S^4T-dR/UVA reflects the production of the 6-4 pyrimidine–pyrimidone-like DNA lesions that have been identified among the products of UVA irradiation of oligonucleotides containing S^4-TdR.[14] However, our observation that XP cells did not show hyper-sensitivity to S^4-BrdU/UVA treatment indicates S^4-BrdU may act by a mechanism different from that of S^4-TdR. This is of potential use regarding effective DNA damage. It is well documented that BrdU (the oxy analogue of S^4-BrdU) can be readily incorporated into cell DNA and its sensitivity to UVA light is dependent on the presence of a photo-sensitiser (i.e., Hoechst dye).[15] A

combination of BrdU and Hoechst dye dramatically sensitises cells to the lethal effect of UVA[16] as the bound dye can absorb UVA photons and provide the energy for dissociation of the bromine atom,[16] which in turn promotes DNA chain breakage. Our synthetic S^4-BrdU itself can act as the primary chromophore and obviate the requirement for the dye. In addition, the bromo group in S^4-BrdU could dissociate itself from its excited state as in the case of BrdU/dye to cause DNA chain breakage. Therefore S^4-BrdU/UVA treatment would have an alternative avenue to execute cell death.

2.4. Chemical properties

S^4-BrdU retains the strong nucleophilicity of the sulfur atom and can be readily functionalised with S_N2-type electrophiles. For instance, monobromobimane (mBB),[17] a thiol-reactive fluorophore for tagging cysteine in peptides, reacts with S^4-BrdU to produce a stable, highly fluorescent compound **4** (Fig. 1). The reaction is confirmed by spectral methods[18] to take place at the S^4-position of the nucleoside. The fluorescent functionalisation of S^4-BrdU is thiol-specific and almost quantitative even in the presence of a large excess of thymidine or BrdU. The marked differences in fluorescence spectra of S^4-BrdU (**3a**) and fluorophore-conjugated-S^4-BrdU (**4**) are demonstrated in Figure 1. The extremely high fluorescence of **4** can offer a sensitive monitoring of minute amounts of S^4-BrdU in treated cells or patients.

3. Summary

We have made the first synthesis of S^4-BrdU and defined some of its important characteristics. Further exploration of its other biological and clinic use is clearly warranted. The synthesis of S^4-BrdU reported here should offer a straightforward route to other 4-thio-5-substituted pyrimidine nucleosides and provide the key precursor for preparation of DNA oligomers containing the modified base. The availability of such modified DNA will facilitate the examination of its coding properties and possible mutagenicity. These studies are now under way.

Acknowledgements

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5. The protection of 3′,5′-hydroxyl groups by silylation was carried out according to a published protocol.[6] The product was directly used for the next step. Pure product was obtained by a silica gel column and characterised by NMR. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.93–2.17 (m, 2H, 2′-H and 2″-H), 3.56∼3.74 (m, 2H, 5′-H), 3.84 (m, 1H, 4′-H), 4.22 (m, 1H, 3′-H), 6.15 (t, J = 6.4, 1H, 1′-H), 8.12 (q, J = 13.1, 1H, 6-H), 8.42 (s, 1H, NH).
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8. **3b** in THF was acidified with 1 N HCl (pH 3) and the reaction monitored by TLC. The desilylated product **3a** was purified by a silica gel column to give pure 4-thio-5-bromo-2′-deoxyuridine. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.17–2.25 (m, 2H, 2′-H and 2″-H), 3.56∼3.65 (m, 2H, 5′-H), 4.09 (m, 1H, 4′-H), 4.22 (m, 1H, 3′-H), 5.21 (t, J = 4.8, 1H, 5′-OH), 5.26 (d, J = 4.4, 1H, 3′-OH), 6.02 (t, J = 6.0, 1H, 1′-H), 8.52 (s, 1H, 6-H), 13.08 (s, 1H, NH). UV: λmax = 340 nm (ε = 17×10^3). HRMS: m/z 322.9699 ([M$^+$ + 1]) (calcd for $C_9H_{12}O_4N_2Br_1S_1$: 322.9696).
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12. MRC5VA cells were grown in a medium containing dialysed fetal calf serum and no added nucleoside for 72 h, then 100 μM S^4-BrdU for 72 h. 10^4 cells were plated per well of a 96 well plate and the incorporated nucleoside detected using a Cell Proliferation ELISA, BrdU (colorimetric) kit (from Roche).
13. The tested cell lines include SV-40 transformed normal MRC5VA fibroblasts, the nuclear excision repair-defective XPA fibroblast XP12RO and its *hMSH2*-defective variant XP12ROB4.
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18. 4-Thio-5-bromo-2′-deoxyuridine (61.54 mg, 0.191 mmol) was dissolved in 0.5 mL of 0.5 M phosphate buffer (pH = 8) and 0.5 mL of CH_3CN. The solution was treated with monobromobimane (50 mg, 0.185 mmol) and stirred at rt for 5 h, by which period, TLC showed that the starting material was completely converted into a new compound with higher R_f [in CH_2Cl_2:CH_3OH (9:1)]. The solution was then mixed with 10 mL of saturated aqueous NaCl and extracted with EtOAc (3×25 mL). The organic extracts were combined, dried over Na_2SO_4, and concentrated under vacuum. Purification of the residue by column chromatography in silica gel (eluting with 5% MeOH/CH_2Cl_2) afforded compound **4** (30 mg, 31%). Data: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.72 (s, 3H, CH_3), 1.89 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.53–3.69 (m, 2H, 5′-H), 3.85 (m, 1H, 4′-H), 4.21 (m, 1H, 3′-H), 4.51 (s, 2H, −SCH_2), 5.24 (m, 2H, 3′ and 5′, −OH), 5.98 (t, J = 5.85 and 5.88, 1H, 1′-H), 8.62 (s, 1H, 6-H); UV: λmax = 263.8 (ε = 12×10^3), 328 (ε = 9×10^3). HRMS: m/z 530.0707 ([M + NH_4]$^+$) (calcd for $C_{19}H_{25}O_6N_5SBr$: 530.0703); Fluorescence: Emission = 440 nm while excited at 380 nm).