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SYNTHESIS OF AN AMINOPROPYL ANALOG OF THE EXPERIMENTAL ANTICANCER DRUG TALLIMUSTINE, AND ACTIVATION OF ITS 4-NITROBENZYLCARBAMOYL PRODRUG BY NITROREDUCTASE AND NADH

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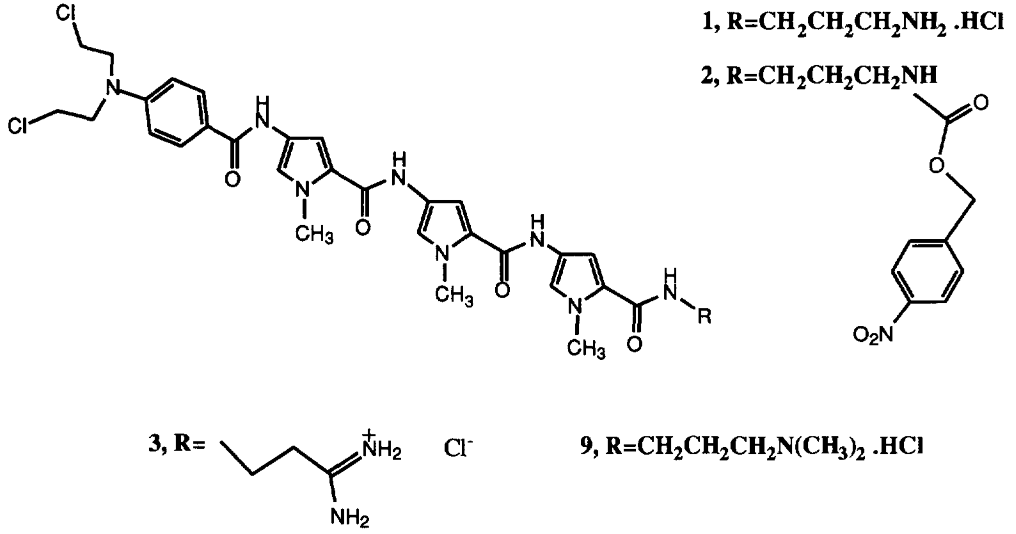
Abstract: Compound **1**, an analog of tallimustine that contains an aminopropyl group at the C-terminus, and its 4-nitrobenzylcarbamoyl prodrug **2** have been prepared. Analog **1** binds preferentially to the minor groove of poly(dA-dT) over poly(dG-dC), and is cytotoxic against the growth of LS1747 and SW1116 human colon cancer cells, with IC_{50} values of 0.02 μM and 10.0 μM, respectively. The prodrug **2** is significantly less cytotoxic (2.3 and 22.9 μM, respectively) than the parent drug **1**. However, in the presence of nitroreductase and NADH prodrug **2** is as active as compound **1**. © 1997 Elsevier Science Ltd.

Tallimustine **3** a N-terminal benzoic acid mustard derivative of distamycin,[1] is presently undergoing phase II clinical trials for cancer treatment in Europe.[2] The results from phase I clinical studies revealed that it was generally well tolerated by patients, and demonstrated some anticancer activity.[2,3] Molecular pharmacological studies of tallimustine have demonstrated that it exerts its biological activity by preferentially reacting with the 3'-adenine-N3 atom of sequences containing the 5'-TTTTPuA-3' site; Pu is either adenine or guanine.[4] Recently, results from our laboratory on analogs of tallimustine that contain either one, two, or three pyrrole units have demonstrated a relationship between covalent sequence specificity, the number of pyrroles, and cytotoxicity.[5]

The primary dose limiting toxicity of tallimustine in the phase I trials was myelotoxicity, suggesting that this compound has poor selectivity for tumor tissues.[3] This problem of poor tumor selectivity is generally observed for most conventional anticancer agents so there is a concerted effort to design molecules, or analogs and derivatives of anticancer drugs that can be delivered and selectively activated at the tumor. A number of drug delivery methods that have been developed include the use of site specific activatable photosensitizers,[6] bioreductively activatable agents,[7] monoclonal antibody-drug conjugates,[8] and antibody directed enzyme prodrug therapy (ADEPT).[9] This latter approach involves targeting tumor tissues by employing covalently bound "foreign" enzymes to tumor specific monoclonal antibodies. After administration of the mAb-enzyme conjugate, a prodrug, an inactive derivative of a highly potent anticancer agent which can be activated by the enzyme, is given to the patient. A number of enzymes have been explored with ADEPT, including nitroreductase, carboxypeptidase G2, and β-lactamase. One ADEPT prodrug designed to be activated by CPG2 is presently undergoing clinical trials, and another is in clinical development.[10]



1, R=$CH_2CH_2CH_2NH_2$ .HCl

2, R=$CH_2CH_2CH_2NH$

3, R=

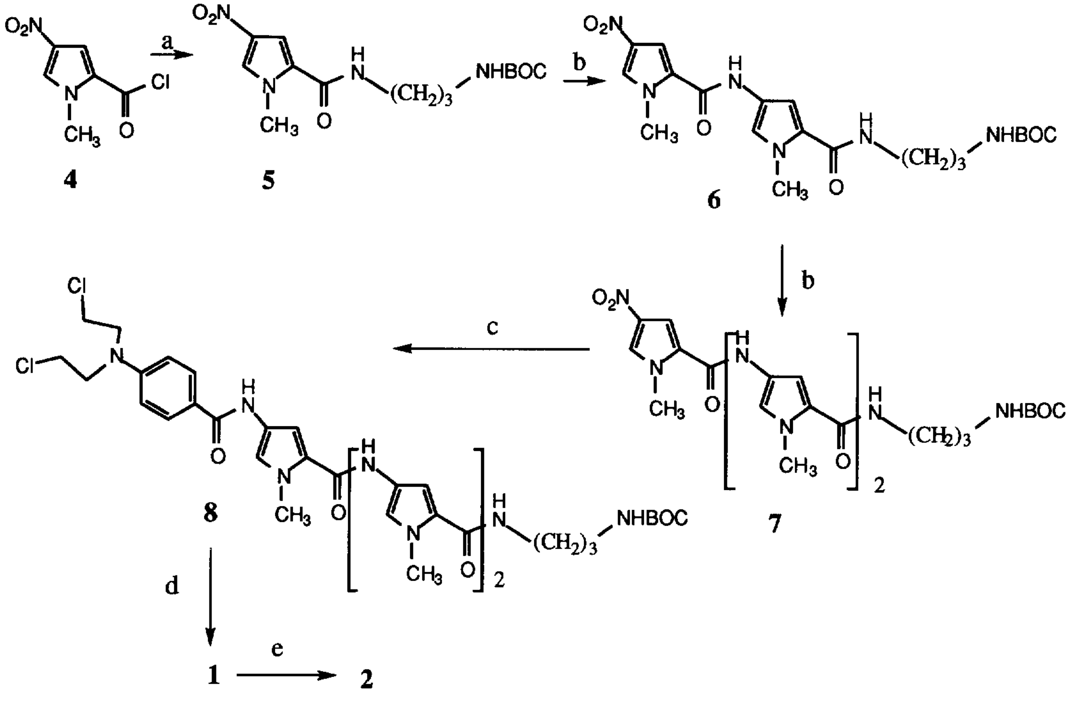
9, R=$CH_2CH_2CH_2N(CH_3)_2$ .HCl

Figure 1. Structures of the target aminopropyl analog **1**, the 4-nitrobenzylcarbamoyl derivative **2**, tallimustine **3**, and its dimethylamino analog **9**.

In this study of targeting tallimustine to tumor tissues using an ADEPT approach, an aminopropyl analog **1** was designed and prepared. In this design, the N-terminus amino function will be protonated at physiological pH of 7.4, and thus will be attracted to DNA. In addition, it can also serve as a nucleophile for derivatization. It is hypothesized that without a basic group in prodrug **2**, it will not bind effectively to DNA and will therefore be less cytotoxic than the parent compound **1**. The enzyme chosen for this study is nitroreductase, a 24kDa flavoenzyme that is capable of using either NADH or NADPH to reduce nitroaromatic groups to their hydroxylamine products.[11] For prodrug **2**, conversion to the hydroxylamine will result in self-immolation of the product, and release the toxic drug, **1**. Nitroreductase has been used to activate a number of other DNA interactive agents, including nitrogen mustards[12] and enediynes.[13]

Results and Discussion:

The syntheses of analog 1 and its prodrug 2 were accomplished according to Scheme 1. Reaction of 1-methyl-4-nitropyrrole-2-carbonyl chloride 4[14] with N-BOC-1,3-diaminopropane in the presence of triethylamine gave amide 5 in 81% yield. Reduction of the nitro moiety in compound 5 by catalytic hydrogenation followed by coupling of the resulting amine with an equivalent of acid chloride 4, in the presence of pyridine, provided the dipyrrole compound 6 in 70% yield. A subsequent cycle of reduction-coupling on compound 6 gave the tripyrrole compound 7 in 71% yield. Catalytic hydrogenation of compound 7 followed by coupling with



Scheme 1. [a]N-BOC-1,3-diaminopropane, dry CH_2Cl_2, dry triethylamine, 0 °C to rt, 16 h. [b](i) H_2, 5% Pd-C, MeOH, rt. (ii) acid chloride 4 in dry CH_2Cl_2, added to a solution of amine in dry pyridine at 0 °C, warmed to rt over 16 h. [c](i) H_2, 5% Pd-C, MeOH, rt. (ii) *p*-*N*,*N*-bis(-2-chloroethyl)aminobenzoyl chloride in dry CH_2Cl_2, added to a solution of amine in dry CH_2Cl_2 containing dry triethylamine at 0 °C, warmed to rt over 14 h. [d]Dry MeOH, HCl (g), 0 °C, 2 h, [e]4-nitrobenzyl chloroformate, 4 M NaOH (2 equiv), THF, water, 0 °C to rt, 1.5h.

p-N,N-bis(-2-chloroethyl)aminobenzoyl chloride[15] gave mustard **8** in 16% yield. Removal of the BOC group was achieved by treatment of **8** with anhydrous methanolic HCl at 0 °C, giving compound **1** in quantitative yield. The 4-nitrobenzylcarbamoyl prodrug **2** was obtained by reaction of compound **1** with 4-nitrobenzyl chloroformate in the presence of sodium hydroxide. The structures of all compounds were confirmed by 300 MHz ^{1}H NMR, FTIR, FABMS, and accurate mass measurements.[16]

The ability of compound **1** to bind reversibly to DNA was studied using an ethidium bromide displacement assay.[17] The apparent DNA binding constants of this compound for calf thymus DNA, coliphage T4 DNA, poly(dA-dT) and poly(dG-dC) given in Table 1 are compared to those of compound **9**, an extensively studied dimethylaminoethyl analog of tallimustine.[5] The results indicate that analog **1** has similar noncovalent DNA binding properties to compound **9**. First, they both demonstrate a preference for poly(dA-dT) over poly(dGdC). Second, because of their strong binding to T4 DNA, whose major groove is occluded by α-glycosylation,[18] these compounds are most likely binding in the minor groove.

Table 1. Apparent DNA Binding Constants (x 10^5 M^{-1})[a]

Compound	Calf Thymus	T4	Poly(dA-dT)	Poly(dG-dC)
1	91.2 (0.5)	217 (0.5)	83.7 (0.7)	52.5 (0.8)
9[b]	37.0	87.0	26.0	71.0

[a]Errors are given in the parentheses. [b]Taken from 5.

The preference of compound **1** for poly(dA-dT) over poly(dG-dC) was corroborated by CD titration studies, where a positive dose-dependent increase in a DNA-induced ligand band at 345 nm was observed for poly(dA-dT) (data not shown). Because compound **1** does not produce a CD spectrum, the appearance of a DNA-induced ligand band is indicative of its binding to the polynucleotide. At an r' (i.e., moles of ligand to moles of DNA base pairs) of 0.20, the DNA-induced band was 5.0 mdeg. For comparison, virtually no DNA-induced band was observed for the addition of compound **1** to poly(dG-dC), even to an r' of 1.0.

A CD dilution method was used to measure the ability of agent **1** to bind irreversibly to DNA.[1a] In this assay, a mixture of compound **1** (r' = 0.35) with poly(dA-dT) was incubated at 37 °C for 16 h. The CD spectra of this mixture taken before and after dilution with 1% SDS revealed that 67 ± 5% of the ligand was bound irreversibly to the polynucleotide, presumably by covalent reaction between a chloroethyl group and an adenine-N3. For comparison, analog **9** was found to produce 17% of irreversible binding to calf thymus DNA.[5] These results suggest that modification of the C-terminus does not significantly affect the DNA binding properties of the agents. Further detailed analysis of the covalent sequence specificity of compound **1** will be reported separately.

Both compound **1** and its prodrug **2** were subjected to in vitro cytotoxicity studies against the growth of LS174T and SW1116 human colon cancer cells. The cells were treated for one hour, and cytotoxicities were determined using a sulphorhodamine B assay.[19] The results given in Table 2 show that the prodrug is deactivated by addition of the 4-nitrobenzylcarbamoyl group at the C-terminus by >100-fold. It is, however, a substrate for nitroreductase, and in the presence of the enzyme and NADH generates equivalent cytotoxicity to the parent compound **1**. Both compounds **1** and **2** are less cytotoxic towards SW1116 cells than the LS174T cells. Our studies also showed that prodrug **2** required both the enzyme and NADH to be activated, otherwise its cytotoxicity was similar to the prodrug alone (data not shown). These data demonstrate the feasibility of delivering derivatives of tallimustine to tumor sites using an ADEPT approach.

Table 2. Cytotoxicities of compound **1** and its prodrug **2**.

Compound	Cells	IC50 (μM)[a]	IC50 (NR + NADH)[b]
1	LS174T	0.02	not done
1	SW1116	10.0	not done
2	LS174T	2.3	0.02
2	SW1116	22.9	9.4

[a] Each IC50 value is the average of two measurements. [b] NR is nitroreductase.

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16. Compound **1**: off-white powder; mp 195 - 208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 1.79 (quintet, 5.9, 2H), 2.80 (m, 2H), 3.25 (q, 6.0, 2H), 3.82 (m, 11H), 3.75 (s, 3H), 3.82 (s, 3H), 6.83 (d, 9.0, 2H), 6.96 (d, 1.8, 1H), 7.04 (d, 1.8, 1H), 7.10 (d, 1.8, 1H), 7.21 (d, 1.8, 1H), 7.25 (d, 1.8, 1H), 7.30 (d, 1.8, 1H), 7.87 (d, 9.0, 2H), 7.94 (s br, 3H), 9.93 (s, 1H), 9.96 (s, 1H), 10.04 (s, 1H) ppm; IR (KBr) 3335, 2910, 1638, 1605, 1513, 1458, 1376, 1272 cm^{-1}; FABMS (NBA) *m/z* 684 (M+H$^+$, 2). Accurate mass for $C_{32}H_{40}N_9O_4{}^{35}Cl_2$: calcd. 684.2580, obsd. 684.2579.

    Compound **2**: off-white powder; mp 119 - 121 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 1.63 (quintet, 5.7, 2H), 3.05 (m, 2H), 3.19 (q, 5.7, 2H), 3.79 (m, 11H), 3.86 (s, 3H), 3.88 (s, 3H), 5.12 (s, 2H), 6.80 (d, 9.0, 2H), 6.95 (s, 1H), 6.97 (s, 1H), 7.15 (s, 1H), 7.21 (s, 1H), 7.25 (s, 1H), 7.36 (s, 1H), 7.55 (d, 9.0, 2H), 7.78 (d, 9.0, 2H), 7.91 (s, 1H), 7.96 (s, 1H), 8.18 (d, 9.0, 2H), 9.83 (s, 1H), 9.86 (s, 1H), 10.03 (s, 1H) ppm; IR (KBr) 3327, 2964, 1634, 1600, 1515, 1435, 1259, 1098, 1018, 799 cm^{-1}; FABMS (NBA) *m/z* 863 (M+H$^+$, 1). Accurate mass for $C_{40}H_{45}N_{10}O_8{}^{35}Cl_2$: calcd. 863.2799, obsd. 863.2795
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