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A NOVEL NITRO-SUBSTITUTED *seco*-CI: APPLICATION AS A REDUCTIVELY ACTIVATED ADEPT PRODRUG

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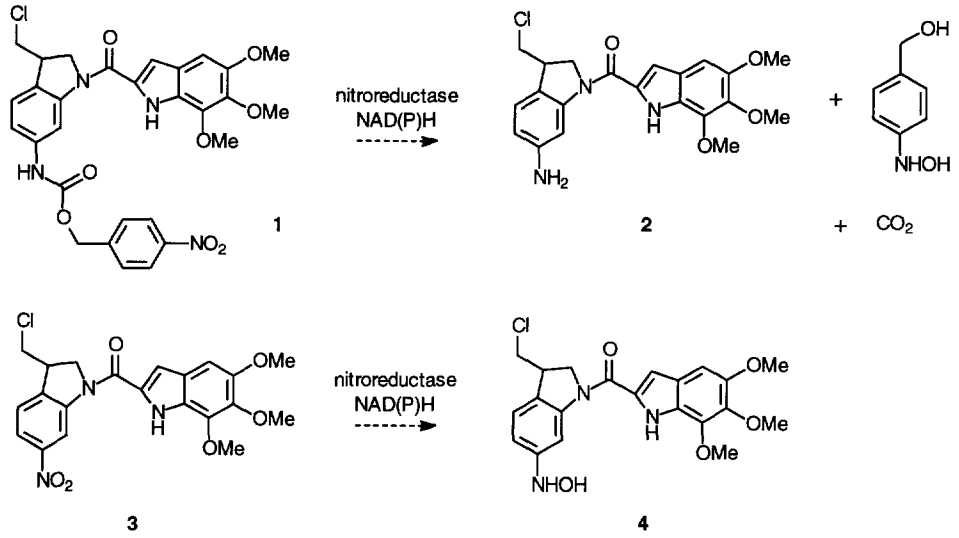
Abstract: An alternative synthesis of *seco*-CI alkylating agents bearing a nitrogen substituent at C-6 is reported. The nitro compound **3** prepared by this route exhibits a 400-fold increase in cytotoxicity against UV4 cells in the presence of a nitroreductase enzyme from *Escherichia coli* B, suggesting it as a potential prodrug for use in antibody-directed enzyme prodrug therapy. Copyright © 1996 Elsevier Science Ltd

Antibody-directed enzyme prodrug therapy (ADEPT) is a new technique with promising potential in cancer chemotherapy. In this approach a tumor specific antibody-enzyme conjugate is administered, followed by a prodrug that is selectively activated by the enzyme at the surface of antigen-expressing tumor cells. A number of ADEPT systems are in various stages of development and have been reviewed.[1] One enzyme under evaluation is an aerobic nitroreductase from *Escherichia coli* B, which in conjunction with NADH or NADPH, reduces certain aromatic nitro groups to the corresponding hydroxylamines.[2] Known substrates for this nitroreductase include CB 1954 (5-aziridinyl-2,4-dinitrobenzamide)[3] and several 4-nitrobenzyloxycarbonyl derivatives, including those of actinomycin D and mitomycin C, which are activated by reductive fragmentation of the nitrobenzyloxycarbonyl group.[4] As prodrugs for ADEPT, these substrates have some limitations, particularly with regard to their enzyme kinetics and the potency of the released cytotoxin. As part of a programme to prepare improved nitroreductase prodrugs we have recently described promising results with a series of dinitrobenzamide mustards[5] and a 4-nitrobenzyloxycarbonyl enediyne derivative.[6] We now wish to report two new prodrug candidates based on amino derivatives of *seco*-CI alkylating agents.

In the preceding communication[7] we described the synthesis of the first *seco*-CI alkylating agents (derived from the CC-1065 and duocarmycin group of natural products) with heteroatom substitution at the crucial phenol position. Aniline **2** (Scheme 1), although 50- to 120-fold less cytotoxic than the corresponding phenol, retains submicromolar potency, and the possibility existed, by analogy with the examples described above, to prepare a nitroreductase prodrug such as **1**. Preparation of carbamates of phenol *seco*-CI alkylating agents, such as carzelesin[8] and KW 2189 and its analogues[9] is known to decrease the in vitro cytotoxicity by up to 1000 fold or more. We were also intrigued by the possibility of using **3** as a nitroreductase prodrug. In this more speculative proposal activation may arise on reduction of the strongly electron withdrawing NO_2 group of **3** (σ_p 0.78)[10] to the electron donating hydroxylamine **4** (σ_p -0.34),[10] thus increasing the ability of the aromatic ring to participate in an Ar-3' cyclisation.

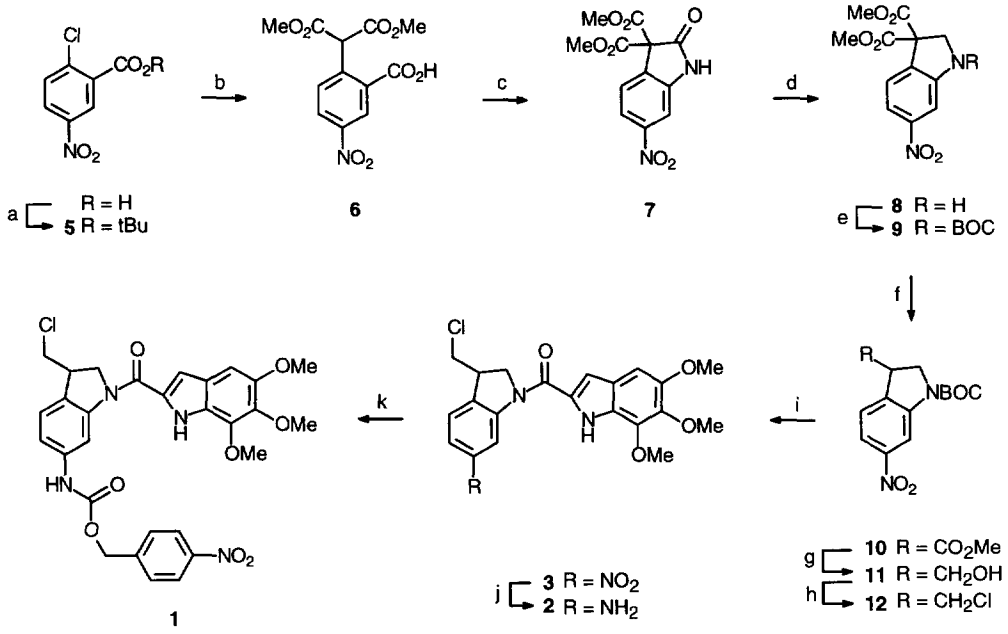
Scheme 1



The synthesis shown in Scheme 2 is an alternative route to racemic amino *seco*-CI compounds, beginning with an isomer of the starting material used in our previous synthesis.[7] The nitro group is carried intact through the synthesis up to the stage of **3**, a compound not readily available from our previous route. Thus, 2-chloro-5-nitrobenzoic acid is converted to its *t*-Bu ester, the chloride displaced by dimethyl malonate anion, and the *t*-Bu ester selectively hydrolysed. Curtius rearrangement of the resulting acid **6** using DPPA (diphenylphosphoryl azide)[11] and triethylamine yielded the lactam **7** through intramolecular trapping of the intermediate isocyanate. Selective reduction of the lactam was possible using borane-dimethyl sulfide, giving indoline **8** in moderate yield, the nitrogen of which was protected using di-*t*-butyldicarbonate. Conversion of diester **9** to the monoester **10** can be achieved under mild conditions because of the capacity of the anion of **10** to act as a leaving group. Accordingly, treatment of **9** with one equivalent of NaOMe at room temperature immediately gives rise to a deep purple nitronate solution; quenching with CF_3CO_2H provides **10** in quantitative yield. Reduction of ester **10** with DIBAL (di-*i*-butylaluminium hydride), conversion to the chloromethyl compound **12**, and deprotection/coupling with 5,6,7-trimethoxyindole-2-carboxylic acid under standard conditions[12] proceeded as expected to give the prodrug candidate **3**. Hydrogenation of **3** over PtO_2 provided **2** in quantitative yield, and further reaction with 4-nitrobenzylchloroformate produced the second prodrug candidate **1**. This alternative synthesis of amino *seco*-CI compounds is an improvement over our previous method[7] in that **2** is now obtained in an overall yield of 16-19% via a route more amenable to multigram scale synthesis.

The ability of the *E. coli* nitroreductase to activate the prodrugs was assayed by determining their cytotoxicity to the Chinese hamster fibroblast-derived cell line UV4 using a published protocol.[5] Thus, cells

Scheme 2



Reagents: (a) $SOCl_2$, DMF then KOtBu (81%) (b) $CH_2(CO_2Me)_2$, NaH then HCO_2H (88%) (c) $(PhO)_2PON_3$, Et_3N (84%) (d) BH_3.DMS (55%) (e) $(BOC)_2O$, DMAP (99%) (f) NaOMe then CF_3CO_2H (g) iBuAlH, THF (87% from **9**) (h) MsCl, Et_3N then LiCl, DMF (92%) (i) HCl then EDCI.HCl, 5,6,7-trimethoxyindole-2-carboxylic acid (60-74%) (j) H_2, PtO_2, THF (100%) (k) p-$NO_2C_6H_4CH_2OCOCl$ (77%)

were exposed for 18 h under aerobic conditions to drug alone, drug and cofactor (1 mM NADH), or drug, cofactor and highly purified enzyme (1 μg/mL), and IC_{50} values calculated after cell growth for a subsequent 72 h (Table). Exposure to drug alone confirms **2** to be a highly cytotoxic species, and that considerable deactivation (>600-fold) is achieved on forming the carbamate **1**. In comparison to **2**, the nitro *seco*-CI **3** is only 100 times less cytotoxic, although this figure is significantly larger (500-fold) for a shorter (4 h) drug exposure. As expected, **2** shows no change in cytotoxicity when administered in the presence of NADH or nitroreductase. Carbamate **1** also shows no change in cytotoxicity with NADH (within experimental error), but is considerably more toxic when the enzyme is present as well. However, the cytotoxicity ratio of 23 is not large; previous values from this assay include 11 (CB 1954),[5] 90 (enediyne derivative),[6] and a maximum of 214 (a dinitrobenzamide mustard),[5] suggesting that this 4-nitrobenzyloxycarbonyl prodrug is not a particularly good substrate for the *E. coli* nitroreductase. Striking results are observed with the nitro compound **3**. In the presence of NADH cytotoxicity increases 10 times, and with the addition of the nitroreductase, **3** is activated

400-fold, to the extent that it appears even more cytotoxic than **2**. The cytotoxic species presumably produced from **3**, the hydroxylamine **4**, is not yet available for direct comparison.

Table

Compound	IC_{50} (nM)[a]			Ratio[b]
	drug alone	drug + NADH	drug + NADH + enzyme	
2 (NH_2)	23.6 ± 3.4	26.6 ± 0.2	27.8 ± 8.8	0.9 ± 0.2
1 (carbamate)	15000 ± 3200	11300 ± 4500	598 ± 140	23.2 ± 2.6
3 (NO_2)	2310 ± 300	237 ± 45	6.4 ± 0.7	402 ± 41

(a) IC_{50} against UV4 cells for 18 h drug exposure in the presence and absence of the *E. coli* B nitroreductase and cofactor. Values are average ± sem for 2-5 determinations. (b) IC_{50} (drug alone)/IC_{50} (drug + NADH + enzyme), intra-experiment ratios.

These data suggest that nitro *seco*-CI compounds have considerable potential as ADEPT prodrugs for use with the *E. coli* nitroreductase. Not only are the cytotoxic species amongst the most potent produced in conjunction with this enzyme, but the cytotoxicity ratio of 400-fold is the highest observed to date in this enzyme assay. The preparation of analogues of **3**, study of their interaction with DNA, and further evaluation is in progress.

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