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A higher yielding synthesis of the clinical prodrug ZD2767P using di-protected 4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl chloroformate

Dan Niculescu-Duvaz, Ian Scanlon, Ion Niculescu-Duvaz and Caroline J. Springer*

Cancer Research UK Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

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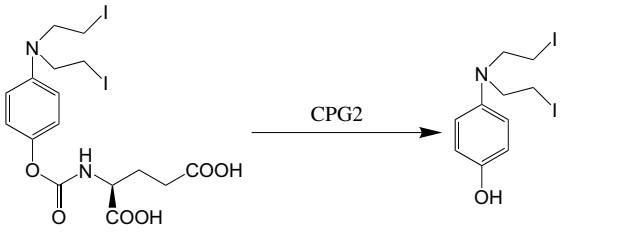
Abstract—A novel synthesis is described of the prodrug ZD2767P (in Phase I/II clinical trials) that improves the overall yield from 13% to 45%. The method involves the synthesis of 4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyl chloroformate protected as the bis-silyl ether, coupled with di-*tert*-butyl glutamate. There are clear advantages of this method compared to the literature procedure.

Antibody-directed enzyme prodrug therapy (ADEPT)[1] and gene-directed enzyme prodrug therapy (GDEPT)[2] are novel strategies to target tumours. Tumour-selective delivery of a foreign enzyme to the tumour is achieved using an antibody–enzyme fusion protein in ADEPT or a vector carrying the enzyme gene in GDEPT. The foreign enzyme activates a relatively non-toxic prodrug to a cytotoxic drug, thereby generating a high local concentration of drug in the tumour, thus minimising side effects to healthy tissues.

Several enzyme–prodrug systems have been described.[3] Carboxypeptidase G2 (CPG2), an enzyme of bacterial origin, has been shown to catalyse the cleavage of an amide, carbamate or urea linkage between glutamic acid and an aromatic group. Based on this specificity, a large number of prodrugs have been designed and synthesised for CPG2.[3–5]

The prodrug ZD2767P (*N*-{4-[*N*,*N*-bis(2-iodoethyl)-amino]phenyloxycarbonyl}-L-glutamic acid **1**) is activated by CPG2 to the alkylating drug 4-[*N*,*N*-bis-(2-iodoethyl)amino]phenol (Scheme 1).[6–11] CPG2 in conjunction with several of the synthesised prodrugs was assessed in ADEPT[9] and GDEPT.[11] ZD2767P



1 Prodrug: (**ZD2767P**)

Drug: 4-[*N*,*N*-bis(2-iodoethyl)amino]phenol

Scheme 1.

was found to possess the best profile in terms of enzymatic kinetics, cytotoxicity differential between CPG2-expressing and non-expressing cell lines and overall in vivo efficacy, and was selected as the clinical candidate for ADEPT with CPG2 (three Phase I/II clinical trials).[12–15]

Several methods[6,7,16] have been described for the synthesis of ZD2767P. In the method described by Heaton et al. (Scheme 2),[16] the first three steps are usually low yielding (4-nitrophenyl chloroformate coupling, reduction of the nitro-derivative to an amine and hydroxyethylation). Nitrophenyl chloroformate coupling to di-*tert*-butyl glutamate is also associated with the formation of variable quantities of symmetric urea of the di-*tert*-butyl glutamate. The overall yield of the method described is 13%.

Keywords: Antibody-directed enzyme prodrug therapy; Prodrug; Scale-up synthesis; Chloroformate; Silyl protection.
* Corresponding author. Tel.: +44 (0)20 8722 4214; fax: +44 (0)20 8722 4046; e-mail: caroline@icr.ac.uk

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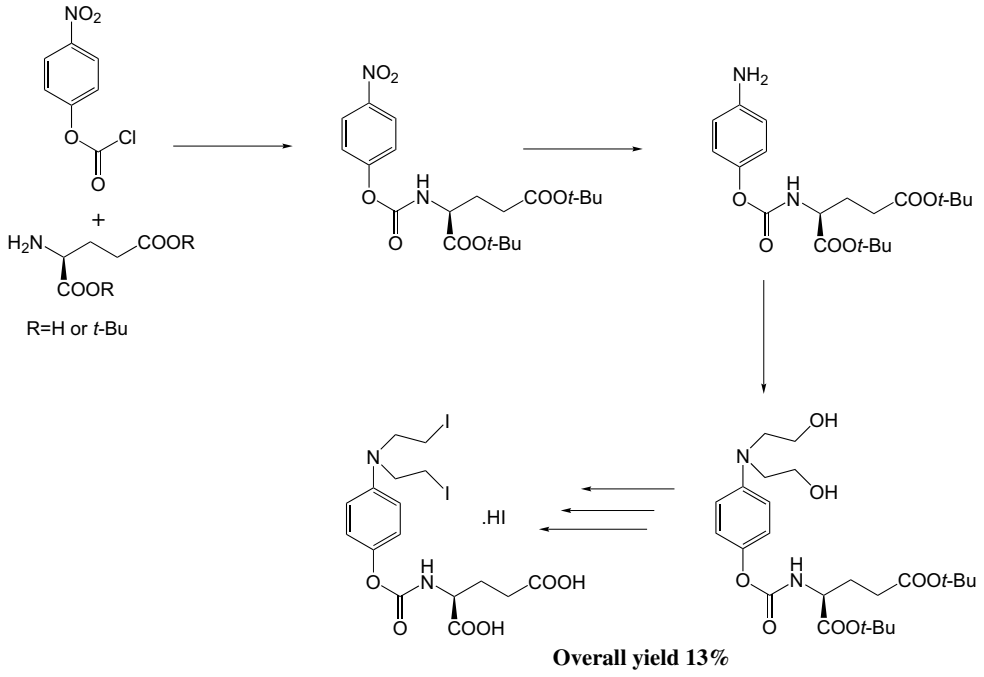


Overall yield 13%

Scheme 2. Synthesis according to Ref. 16.

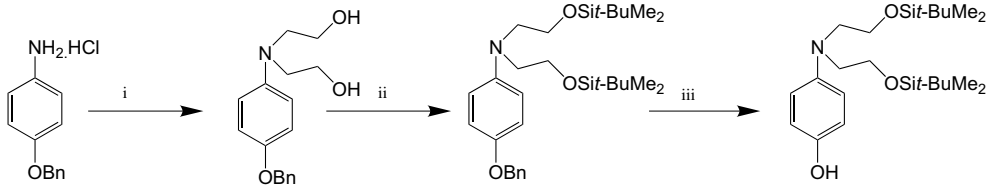
Large quantities of the prodrug were required for the preclinical development of ZD2767P. The low yield of the reported method made it unsuitable for large-scale synthesis. Here, we describe a new method for the synthesis of the key intermediate di-*tert*-butyl*N*-{4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenyloxycarbonyl}-L-glutamate **7** avoiding the pitfalls associated with the previous methods.

4-[*N*,*N*-Bis(2-(*tert*-butyldimethylsilyloxy)ethyl)amino]-phenol **2**, the starting material for coupling with di-*tert*-butyl glutamate **5**, was obtained in almost 80% overall yield from 4-benzyloxyaniline **10**, by hydroxyethylation with ethylene oxide (84%), protection of diol **11** as the *tert*-butyldimethylsilyl ether with the corresponding silyl chloride and imidazole in DMF (95% after purification),[17] and then deprotection of the phenolic benzyl ether **12** by catalytic hydrogenation (Scheme 3).[18]

The phenol **2** was converted into the corresponding chloroformate **3** with phosgene in toluene, followed by coupling with **5** to afford **6** in 90% yield.[19] The bis-silyl ether **6** was deprotected cleanly with $Et_3N{\cdot}3HF$ (yield 97%), and after a basic aqueous work up, the key intermediate **7** was obtained without the need for further purification (Scheme 4).[20]

The bis-hydroxy compound **7** was converted to into its bis-mesyl derivative **8** with mesyl anhydride in dichloromethane. After aqueous extraction with citric acid, **8** was treated with NaI in refluxing acetone to afford **9** (85% from **7**, after purification). The final deprotection with TFA and recrystallisation from toluene/ethyl acetate yielded **1** (90%, Scheme 4). The overall yield starting from glutamic acid **4** was 45%.

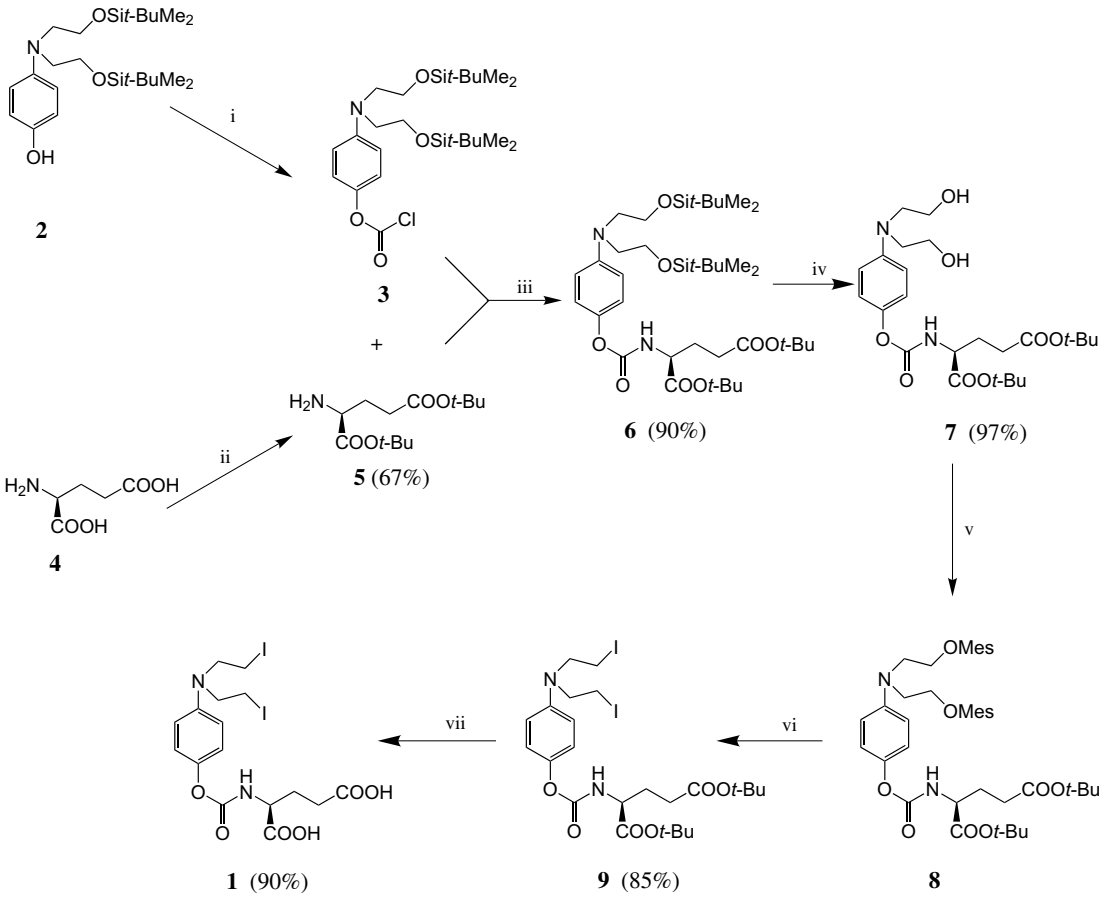
This procedure has several advantages over the previously described method. The overall yield (45%) is more than 3-fold higher compared to the yield of the reported procedure (13%).[16] Also, the synthesis of the key intermediate **7** is much improved, avoiding the low yielding steps of the published method. Another advantage is that the coupling of **3** with **5** is reliable and proceeds with higher yield than the coupling of 4-nitrophenyl chloroformate, which often produces ureas as by-products. In our procedure, the hydroxyethylation is per-



10 **11** (84%) **12** (95%) **2** (100%)

Overall yield 80%

Scheme 3. Reagents and conditions: (i) ethylene oxide, Et_3N, AcOH; (ii) *t*-$BuMe_2SiCl$, imidazole, DMF; (iii) H_2, Pd/C, THF.



Scheme 4. Reagents and conditions: (i) $COCl_2$, Et_3N, toluene; (ii) isobutylene, H_2SO_4, $CHCl_3$; (iii) Et_3N, THF; (iv) $Et_3N{\cdot}3HF$, THF; (v) Mes_2O, Et_3N, DMAP, CH_2Cl_2; (vi) NaI, acetone, reflux; (vii) TFA.

formed on 4-benzyloxyaniline **10** (Scheme 3) with a yield up to 84% and simplified work-up, rather than on the expensive advanced intermediate di-*tert*-butyl *N*-(4-amino-phenoxycarbonyl)-L-glutamate. An additional advantage of the process is the introduction of the di-*tert*-butyl glutamate at a later step, making economies in the use of this starting material.

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17. 4-Benzyloxy-*N*,*N*-bis(2-(*tert*-butyldimethylsilyloxy)ethyl)-aniline **12**: A mixture of 4-benzyloxy-*N*,*N*-bis(2-hydroxyethyl)aniline (9.6 g, 33.4 mmol), *tert*-butyldimethylsilyl chloride (12.7 g, 84 mmol) and imidazole (7.8 g, 130 mmol) was dissolved in dry DMF (80 mL) and the reaction stirred overnight, then the solvent was evaporated, the residue taken in dichloromethane, the precipitated imidazole hydrochloride filtered off, and the filtrate evaporated. The residue was purified by column chromatography on silica (cyclohexane/ethyl acetate 4:1), to afford **12** (16.4 g, 95%) as an oil. ^{1}H NMR δ_H: −0.01 (s, 12H, $2\times SiMe_2$), 0.84 (s, 18H, $2\times Si$*t*-Bu), 3.39 (t, 4H, N($CH_2CH_2OSi)_2$, $J = 6.01$ Hz), 3.67 (t, 4H, N(CH_2CH_2-$OSi)_2$, $J = 5.99$ Hz), 4.96 (s, 2H, PhCH_2), 6.59 (d, 2H, $H_{arom3+5}$, $J = 9.14$ Hz), 6.82 (d, 2H, $H_{arom2+6}$, $J = 9.10$ Hz), 7.25–7.44 (m, 5H, $H_{arom\ benzyl}$). MS (FAB): 516 (M^++H). Microanalysis ($C_{29}H_{49}NO_3Si_2$) required: C, 67.52; H, 9.57; N, 2.72; found C, 67.09; H, 9.41; N, 2.69.
18. 4-[*N*,*N*-bis(2-(*tert*-butyldimethylsilyloxy)ethyl)amino]phenol **2**: The benzyl ether **12** (11.4 g, 22.1 mmol) was dissolved in THF, Pd/C catalyst (1.6 g) was added and the suspension stirred overnight under H_2 atmosphere. The catalyst was filtered off, and the solvent evaporated to afford **2** (9.4 g, 100%) as an oil. ^{1}H NMR δ_H: 0.00 (s, 12H, $SiMe_2$), 0.84 (s, 18H, Si*t*-Bu), 3.34 (t, 4H, N(CH_2-$CH_2OSi)_2$, $J = 6.09$ Hz), 3.65 (t, 4H, N(CH_2 $CH_2OSi)_2$, $J = 6.06$ Hz), 6.51 (d, 2H, $H_{arom2+6}$, $J = 9.23$ Hz), 6.58 (d, 2H, $H_{arom3+5}$, $J = 9.15$ Hz), 8.46 (s, 1H, OH). MS (FAB): 425 (M^+). Microanalysis ($C_{22}H_{43}NO_3Si_2$) required: C, 62.06; H, 10.18; N, 3.29; found C, 61.69; H, 9.79; N, 3.10.
19. Di-*tert*-butyl *N*-{4-[*N*,*N*-bis(2′-(*tert*-butyldimethylsilyloxy)ethyl)amino]phenoxycarbonyl}-L-glutamate (**6**): To a stirred solution of 4-*N*,*N*-bis(2′-(*tert*-butyldimethylsilyloxy)ethyl)amino-phenol **2** (13.2 g, 32 mmol) in toluene (120 mL), phosgene (20% solution in toluene, 35 mL, 70 mmol) was added at once at room temperature. After 2 min, triethylamine (5.3 mL, 38 mmol) was added dropwise, and the formation of chloroformate **3** was complete in 10 min as detected by IR spectra ($\nu_{OCOCl} = 1784$ cm^{-1}). The solution was filtered and the filtrate evaporated to afford an oil, which was dissolved in THF (80 mL) and used directly in the next reaction. Di-*tert*-butyl glutamate hydrochloride **5** (9.77 g, 33 mmol) was dissolved in ethyl acetate (100 mL) and extracted with aq Na_2CO_3 (100 mL). The organic layer was dried ($MgSO_4$) and evaporated. The oily residue was dissolved in THF (80 mL). The solution of chloroformate **3** in THF was poured over the solution of di-*tert*-butyl glutamate **5** under stirring, at room temperature, followed by triethylamine (4.9 mL, 35 mmol). After 5 min, the reaction was completed, as monitored by IR (the chloroformate stretch $\nu_{OCOCl} = 1784$ cm^{-1} disappeared). The precipitate was filtered off, the solvent evaporated and the residue purified by column chromatography on silica (cyclohexane/ethyl acetate 4:1) to afford **6** (20.45 g, 90%) as an oil. ^{1}H NMR δ_H: 0.00 (s, 12H, $2\times SiMe_2$), 0.84 (s, 18H, $2\times Si$*t*-Bu), 1.39 (s, 9H, COO-*t*-Bu), 1.40 (s, 9H, COO-*t*-Bu), 1.75–2.05 (2m, 2H, CH_2CH(NH)–), 2.32 (t, 2H, CH_2COO, $J = 7.69$ Hz), 3.44 (t, 4H, N($CH_2CH_2OSi)_2$, $J = 5.99$ Hz), 3.70 (t, 4H, N($CH_2CH_2OSi)_2$, $J = 5.68$ Hz), 3.90–4.05 (m, 1H, *CH*(NH)–), 6.61 (d, 2H, $H_{arom2+6}$, $J = 9.18$ Hz), 6.83 (d, 2H, $H_{arom3+5}$, $J = 9.03$ Hz), 7.87 (d, 1H, NH, $J = 7.85$ Hz).
20. Di-*tert*-butyl *N*-{4-[*N*,*N*-bis(2-hydroxyethyl)amino]phenoxycarbonyl}-L-glutamate (**7**): To a solution of **6** (20.45 g, 28.8 mmol) in THF (315 mL) was added $Et_3N{\cdot}3HF$ (42 mL). The solution was stirred at room temperature for 12 h, then the solvent was evaporated, the residue was taken in ethyl acetate (100 mL) and extracted with water (100 mL), aq Na_2CO_3 (100 mL) and water again (100 mL). The organic layer was dried ($MgSO_4$) and evaporated to afford **7** (13.4 g, 97%) as a foamy solid.
All the analytical data were identical with those of compound **7** obtained by the procedure described by the works of Niculescu-Duvaz et al.[5]