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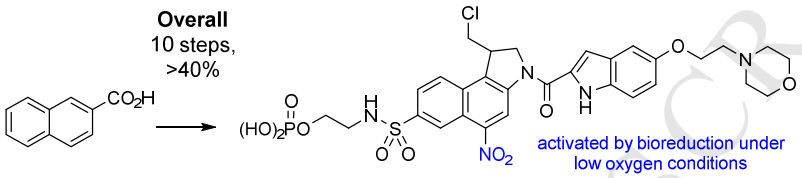

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Optimised synthesis of a nitroCBI hypoxia-activated prodrug with substantial anticancer activity

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ABSTRACT

An optimised synthesis of a hypoxia-activated anticancer prodrug related to the duocarmycin family of natural products is described. The improved 10-step synthesis increases the overall yield from 4.4% to over 40% while requiring just 2 chromatography-based purifications. The most significant improvements optimise the isomer distribution in a key chlorosulfonylation reaction, and facilitate the removal of tin residues from a tributyltin hydride-mediated radical reaction. Improved preparations of two side chains are also reported. The new method provides practical access to sufficient material to support advanced efficacy and toxicology assessments.

1. Introduction

Inspired by the structure and mechanism of action of the duocarmycin natural products[1] (such as duocarmycin SA, **1**, **Figure 1**) we have developed a class of anticancer prodrugs that are selectively toxic to cells under low oxygen conditions.[2–6] The prodrugs are named nitroCBIs (**2**) after the synthetic 1,2,9,9a-tetrahydrocyclopropa[*c*]benz[*e*]indol-4-one (CBI) variant of the alkylating subunit, modified such that the active phenol of the ring-opened form of CBI is replaced with a nitro group.[2,3] In the general structure **2**, R is a side chain that can bind in the minor groove of DNA, and R′ is a substituent that modifies the reduction potential of the nitro group.[3] With appropriate R′ substituents **2** undergoes efficient bioreduction, via an initial 1-electron oxygen-sensitive step, to yield the corresponding aminoCBI **3**. Under physiological conditions **3** spontaneously cyclises to an unstable intermediate **4**[5] that, like the natural products, selectively alkylates the N3 position of adenine in the minor groove of DNA, creating a highly toxic lesion **5** (where dR represents the deoxyribose connection to DNA). Hypoxic cells capable of activating nitroCBIs are not commonly found in normal tissues, but are a prevalent feature of tumours, where they are widely associated with multiple types of treatment resistance.[7] Thus hypoxia-activated prodrugs such as nitroCBIs have great potential to be combined with standard agents to deliver more effective cancer therapy.[8,9]

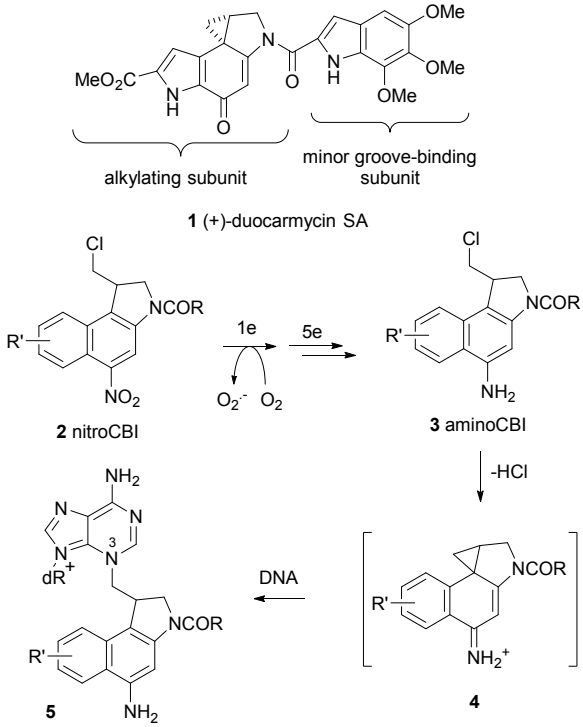

Figure 1: Structure of duocarmycin SA, and mechanism of action of nitroCBI hypoxia-activated prodrugs.

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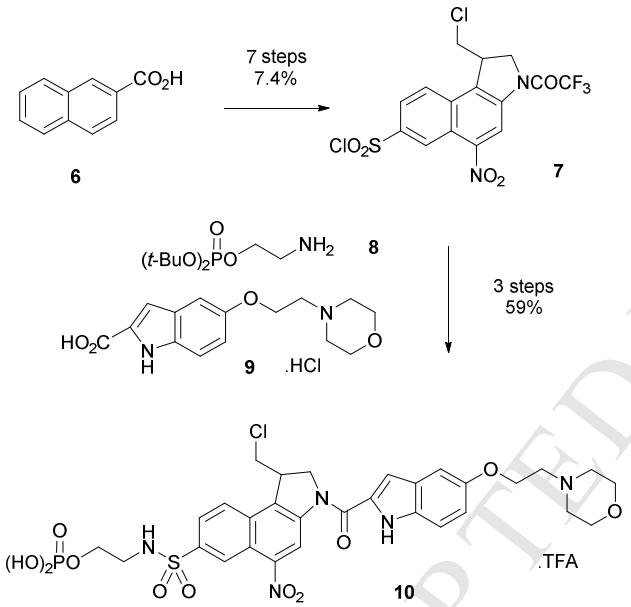
We recently reported a particular nitroCBI **10** (**Scheme 1**) with outstanding anticancer activity.[6] This includes combination with radiotherapy to eliminate all detectable colony-forming cells in some cervical carcinoma xenografts, and combination with chemotherapy (gemcitabine) to cause marked regression of all treated ovarian tumour xenografts. These effects were observed at well-tolerated doses and in some cases gave curative activity (tumour-free mice 100 days after treatment).[6] Our reported synthesis of **10** proceeds in 10 steps and gives an overall yield of only 4.4% (**Scheme 1**). 2-Naphthoic acid **6** is converted to the key intermediate **7** in 7 steps,[3,10] followed by sequential addition of each of the side chains **8**[4] and **9**,[11] and a final deprotection step to give the desired product.[6] We have carefully re-examined each of these steps and have improved the overall yield to >40% (**Scheme 2**) while reducing the number of chromatography-based purifications from 6 to 2. We have also substantially improved the synthesis of each of the side chains (**Scheme 3**), and used the optimised method to prepare multi-gram quantities of **10**. The details of these improvements are described herein, some of which have been disclosed in patent form.[12]



Scheme 1: Overview of the published synthesis of nitroCBI hypoxia-activated prodrug **10**.

2. Results and Discussion

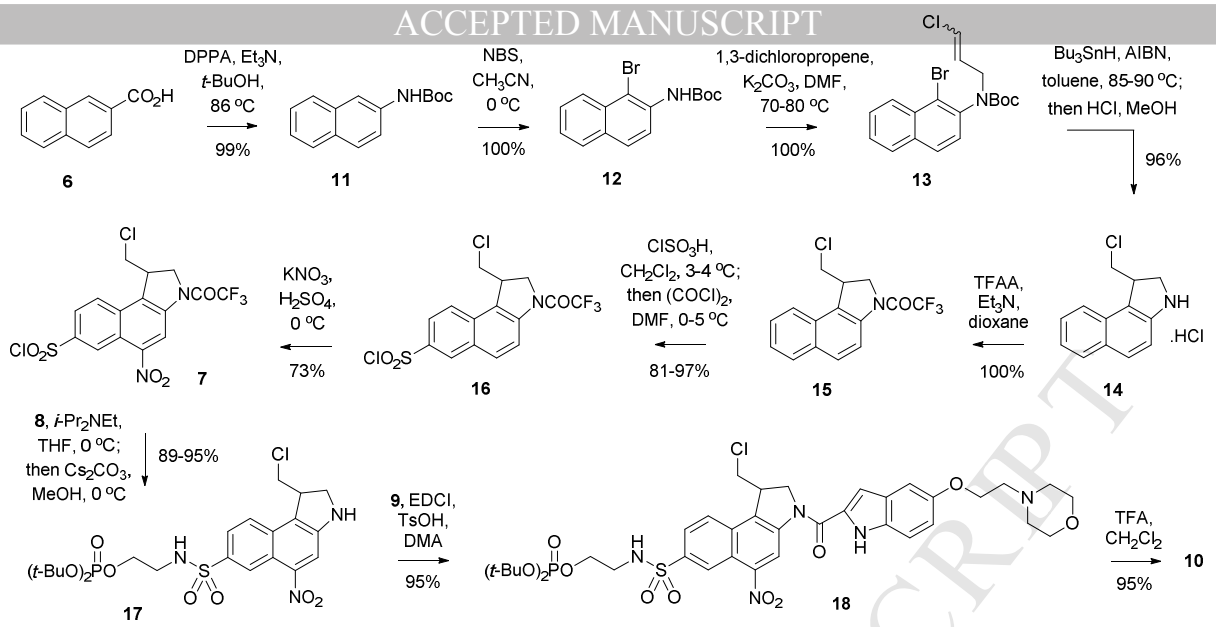
The synthesis begins with the conversion of **6** to **11** using diphenylphosphoryl azide (DPPA) in *t*-BuOH (**Scheme 2**). The procedure was conducted on a much larger scale than previously reported (100 g versus 1.5 g[10]), with minimal excess reagents and solvent, and the crude product isolated in practically quantitative yield without chromatography. This material could be further purified by recrystallisation (75% yield on a 25 g scale), but we found this to be unnecessary to attain high yields in the following steps.

Bromination was achieved simply by exposure to *N*-bromosuccinimide (NBS) at 0 °C in CH_3CN and the product **12** was precipitated from the reaction mixture in quantitative yield. For this step we had found[3] that it was not necessary to use either an acid catalyst or DMAP to facilitate the reaction, in surprising contrast to related duocarmycin analogues bearing an additional and activating benzyloxy substituent.[13,14] For the subsequent chloroallylation step our original method employed NaH as the base, the almost universal choice in multiple related syntheses (with occasional exceptions using KO*t*-Bu[15] or a phosphazene[16]). We successfully scaled up the NaH method (to about 0.5 mol, requiring 25 g of a 60% dispersion of NaH in oil), but later found that it was operationally much simpler to employ K_2CO_3 as the base, which gave quantitative reaction to **13** in DMF at 70-80 °C.

The indoline ring of **14** is formed in a radical-mediated cyclisation reaction. Our previously reported procedure using Bu_3SnH and azobisisobutyronitrile (AIBN) initiator worked well on a large scale (23 g of **13**),[3] but removal of tin residues required 3 purification steps (chromatography, crystallisation from MeOH, and recrystallisation from CH_2Cl_2/petroleum ether) which resulted in a yield of 74%. We modified the procedure by reducing the amount of AIBN used and changing the solvent from benzene to toluene, but the major change was in the purification method. Most of the tin residues were removed by exposure to solid KF (which converts tributyltin halides to insoluble polymeric tributyltin fluoride[17]), followed by filtration through a short column of neutral alumina. The crude product still contained some tin residues (5-10% by ^{1}H NMR) but these were removed after deprotecting the Boc group using methanolic HCl and repeatedly washing the methanol solution with petroleum ether. In this way the hydrochloride salt **14** was obtained in 96% yield starting from nearly 50 g of **13**.

The indoline nitrogen was next protected to give trifluoroacetamide **15** as a substrate for the following chlorosulfonylation reaction. This step was the lowest-yielding of the original synthesis since the reaction, conducted in neat chlorosulfonic acid at 60 °C, gave a mixture of 6- and 7-SO_2Cl isomers, with the desired 7-isomer **16** being the minor component and isolated in only 25% yield after chromatography.[3] We had previously found that the isomer distribution after acetylation of the same substrate **15** was very sensitive to the reaction conditions, favoring the 6-acetyl product when conducted in CS_2 at 70 °C, but the 7-acetyl product in nitrobenzene at room temperature.[3] Therefore we explored alternative conditions for the chlorosulfonylation reaction, and found that using just 1.2 equivalents of $ClSO_3H$ in CH_2Cl_2 at 3-4 °C produced almost exclusively the desired 7-isomer. At this temperature the reaction requires several hours to consume the starting material and during this time an insoluble product appears. On the assumption that this was the corresponding sulfonic acid the mixture was treated with DMF and oxalyl chloride to convert this material back to the desired sulfonyl chloride, a method that eventually produced **16** in up to 97% yield. Occasionally, significant amounts of the 6-SO_2Cl isomer were still produced, requiring purification by column chromatography, or by trituration with EtOAc, but the overall yield was always considerably higher than that previously obtained.

Nitration was achieved using KNO_3 in H_2SO_4, which performed well on a 20 g scale to give the key intermediate **7**. As well as the desired 5-NO_2 isomer small amounts of the 9-NO_2 product were also formed, but this less polar byproduct was easily removed by crystallisation of the crude material from CH_2Cl_2/*i*-Pr_2O. Side chain **8** was introduced via a high-yielding sulfonamide formation, with excess base added to complete removal of the trifluoroacetamide protecting group to generate **17**. Side chain **9** was then appended via amide formation using *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI) as the coupling reagent under weakly acidic conditions. The addition of a second batch of EDCI drove the reaction to completion and gave an excellent yield of **18**. The final step also proceeded in excellent yield, using TFA to remove the *t*-Bu ester protecting groups from the phosphate, leading to the isolation of



Scheme 2: Optimised synthesis of **10**.

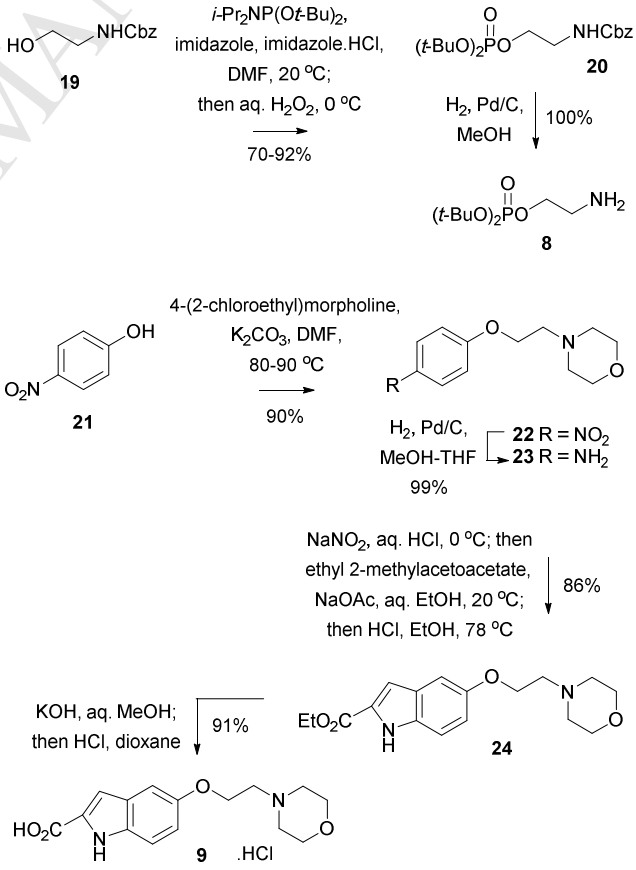
10 as the trifluoroacetate salt. Single batches of up to 3.6 g of **10** have been made following this procedure, with HPLC purity routinely >97%.

Significant improvements in the synthesis of the side chains **8** and **9** are detailed in **Scheme 3**. For **8** the key was to optimize the initial phosphorylation reaction to prepare larger quantities of **20**. We modified our original procedure[4] by reducing the excess of di-*tert*-butyl *N*,*N*-di-*iso*-propylphosphoramidite and tetrazole used, finding the best order for addition of reagents, optimising the reaction time, and by swapping from 70% to 30% H_2O_2 for the oxidation step. These changes gave a crude product from which the desired material could be obtained by crystallisation rather than chromatography. The product **20** is a low mp solid (43-45 °C) and a seed crystal was found to be very useful in inducing the crystallisation process. Even with these carefully optimized conditions, some loss of *t*-Bu groups was occasionally observed by ^{1}H NMR analysis of the crude product. This could represent contamination with a phosphonate byproduct.[18] Switching the acidic activator from tetrazole to a combination of imidazole and imidazole hydrochloride[18] proved to be a useful alternative, providing **20** in up to 92% yield on a multi-gram scale. Hydrogenation then afforded side chain **8**.

The synthesis of side chain **9** proceeds in 4 steps from 4-nitrophenol **21** (**Scheme 3**). The original method[11] used a 2-part procedure for the first step, isolating the potassium salt of **21** and then reacting this with 4-(2-chloroethyl)morpholine in toluene to give **22** in 57% yield. By using K_2CO_3 as the base in DMF this step can be conducted in a one-pot procedure to isolate **22** in 90% yield without chromatography. Following hydrogenation to the aniline **23**, a Japp–Klingemann-type Fischer indole synthesis produced **24**, isolated in 86% yield on a 35 g scale. The final step is a simple ester hydrolysis, for which a minor modification aided purification on a large scale – the product was precipitated and washed in the zwitterionic form before conversion to the hydrochloride salt, allowing the isolation of **9** in high yield, again without recourse to chromatography.

3. Conclusions

Overall these modifications constitute a significantly improved synthesis of a highly promising anticancer prodrug, and provide a practical route for the synthesis of sufficient material to support further efficacy and toxicology evaluation. Some of the modifications may well be applicable to the synthesis of duocarmycin analogues in general, which are of current interest in targeted anticancer therapies such as antibody-drug conjugates.



Scheme 3: Optimised synthesis of side chains **8** and **9**.

4. Experimental Section

4.1. General Experimental

Solvents were distilled prior to use by common laboratory methods. *t*-BuOH was dried over CaH_2. Petroleum ether refers to the fraction with a bp = 40-60 °C. Melting points were determined on an Electrothermal 2300 Melting Point Apparatus. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra. HRMS was performed on an Agilent 6530B Accurate Mass Q-TOF mass spectrometer. Combustion analyses were carried out in the Campbell Microanalytical Laboratory, University of Otago, Dunedin, New Zealand.

4.2. Synthesis

*4.2.1. tert-Butyl 2-naphthylcarbamate (**11**)*

Et_3N (89.2 mL, 638 mmol) was added to solid 2-naphthoic acid (**6**) (100 g, 581 mmol) and the mixture was stirred at 20 °C for 10 min. After this time the mixture solidified and was allowed to stand at 20 °C for a further 20 min. DPPA (freshly opened bottle, 131.6 mL, 609 mmol) was added, followed by dry *t*-BuOH (277.2 mL, 2.90 mol), causing the internal temperature to rise to 35 °C. The reaction flask was placed in an oil bath and the bath temperature was raised from 20 °C to 86-87 °C over 1 h 20 min, then held at this temperature for 2 h. The reaction mixture was cooled briefly and poured into ice-water (ca. 2.5 L) with vigorous stirring at 20 °C, causing a sticky solid mass to separate. The mixture was left to stand at 20 °C overnight (15 h) to give a loose suspended solid. The mixture was stirred again for 5 h. The fine solid was filtered off, washed with water (4 × 300 mL) and dried in a vacuum oven at 40-45 °C to constant weight, to give **11** as a beige solid (140 g, 99%); mp 85-87 °C [lit. mp 92-93 °C (hexane)[19]], suitable (^{1}H NMR) for the following reactions. A portion was recrystallised from CH_2Cl_2/petroleum ether to give a cream solid; δ_H ($CDCl_3$) 7.98 (s, 1 H), 7.75 (apparent d, *J* = 8.6 Hz, 3 H), 7.45-7.41 (m, 1 H), 7.38-7.31 (m, 2 H), 6.60 (br s, 1 H), 1.56 (s, 9 H); consistent with that reported.[10,19]

*4.2.2. tert-Butyl 1-bromo-2-naphthylcarbamate (**12**)*

A stirred solution of **11** (92 g, 0.38 mol) in acetonitrile (680 mL) at 0 °C was treated portionwise over 2 h with solid NBS (81 g, 0.45 mol). After the addition was complete the reaction mixture was stirred for a further 45 min at 0 °C. To the mixture was added a cold solution of aqueous $NaHCO_3$ (0.2N, 2 L) and the mixture was stirred at 0 °C for 1 h. The solid was filtered off and washed with water (5 × 200 mL), then dried in vacuum over solid KOH overnight to give **12** as a beige solid (121 g, 100%); mp 88-89 [lit. mp 88-89 °C;[19] 90-91 °C (MeOH)[3]]; δ_H [$(CD_3)_2SO$] 8.78 (s, 1 H), 8.15 (d, *J* = 8.6 Hz, 1 H), 7.99-7.92 (m, 2 H), 7.71 (d, *J* = 8.8 Hz, 1 H), 7.69-7.64 (m, 1 H), 7.58-7.53 (m, 1 H), 1.49 (s, 9 H); consistent with that reported.[3]

*4.2.3. tert-Butyl 1-bromo-2-naphthyl-(3-chloro-2-propen-1-yl)carbamate (**13**)*

A mixture of **12** (53.7 g, 167 mmol) and dry K_2CO_3 (55.2 g, 400 mmol) in dry DMF (500 mL) was stirred at 20 °C for 5 min, then 1,3-dichloropropene (23.1 mL, 251 mmol, mixed isomers) was added. The mixture was stirred at 70-80 °C (bath temperature) under a dry atmosphere for 4 h. The mixture was cooled and then partitioned between petroleum ether (500 mL) and water (500 mL). The petroleum ether layer was separated and washed with water (3 × 200 mL), then dried (Na_2SO_4) and allowed to stand at 5 °C overnight. The precipitated orange solid was filtered off and the filtrate was evaporated to give an amber oil (67.5 g). This was again dissolved in petroleum ether and the procedure repeated (precipitation of an orange solid, filtration, evaporation) to give **13** as an oil (66.2 g, 100%), suitable (^{1}H NMR) for the following reactions; δ_H [$(CD_3)_2SO$] (mixture of rotamers and *E* and *Z* forms) 8.23 (d, *J* = 8.4 Hz, 1 H), 8.05-7.97 (m, 2 H), 7.74-7.62 (m, 2 H), 7.50, 7.45 (2 d, *J* = 8.6 Hz, 1 H), 6.41-6.30 (m, 1 H), 6.18-6.02 (m, 1 H), 4.57-4.49, 4.42-4.19, 4.10-3.97 (3 m, 2 H), 1.49, 1.26 (2 s, 9 H); consistent with that reported.[3]

*4.2.4. 1-(Chloromethyl)-1,2-dihydro-3H-benzo[e]indole hydrochloride (**14**)*

A solution of **13** (48.8 g, 123 mmol) in toluene (150 mL) was stirred at 20 °C under nitrogen for 20 min, then Bu_3SnH (34.2 mL, 123 mmol) and AIBN (60.7 mg, 0.37 mmol) were added. The reaction mixture was stirred at 85-90 °C (bath temperature) under nitrogen for 1 h and then cooled to 20 °C. Powdered anhydrous KF (35.7 g, 615 mmol) was added and the mixture was stirred at 20 °C for 14 h. EtOAc (150 mL) and petroleum ether (300 mL) were added and the mixture stirred for a further 1 h 30 min. The mixture was filtered through a pad of neutral alumina (300 g), washing with EtOAc/petroleum ether (1:2) (10 × 50 mL). The combined filtrates were washed successively with water (300 mL), aqueous Na_2CO_3 (5%, 300 mL) and water (300 mL), and then dried (Na_2SO_4) and evaporated to give a yellow solid (74 g). ^{1}H NMR analysis showed this material contained about 5-10% tin residues. The solid was dissolved in MeOH (150 mL) and HCl gas was bubbled into the solution at room temperature over 25 min. The acidic mixture was stirred for a further 10 min, then diluted with petroleum ether (300 mL). The mixture was stirred at 20 °C for 15 min, then the petroleum ether layer was separated and discarded. The petroleum ether procedure was repeated three more times. The acidic MeOH solution was then evaporated under reduced pressure at 35-40 °C (bath temperature) to give **14** as a greenish foamy solid (30.1 g, 96%); mp 180 °C (dec.); δ_H [$(CD_3)_2SO$] ~10.5 (v br, 1 H), 8.04-7.94 (m, 3 H), 7.66-7.60 (m, 1 H), 7.56-7.51 (m, 1 H), 7.45 (d, *J* = 8.7 Hz, 1 H), 4.43-4.36 (m, 1 H), 4.08 (dd, *J* = 11.0, 3.5 Hz, 1 H), 3.96 (d, *J* = 11.2 Hz, 1 H), 3.93 (d, *J* = 11.2 Hz, 1 H), 3.84 (dd, *J* = 11.5, 2.5 Hz, 1 H). This material contained only traces of tin residues (about 1%). On a smaller scale (3.0 g, Boc deprotection conducted using 4 M HCl in dioxane) repeated washes with petroleum ether (4 × 100 mL) were sufficient to remove all traces of tin residues (as detected by ^{1}H NMR) to give an analytically pure product; δ_C [$(CD_3)_2SO$] 137.1, 132.3, 130.5, 129.1, 129.0, 127.8, 126.1, 123.8, 116.7, 48.7, 46.3, 43.3 (one aromatic quaternary carbon not observed); [Found: C, 59.71; H, 5.36; N, 5.32. $C_{13}H_{12}ClN{\cdot}HCl{\cdot}½H_2O$ requires C, 59.33; H, 5.36; N, 5.32]; HRMS (ESI) $MH^{+\cdot}$ found 218.0727. $C_{13}H_{13}ClN$ requires 218.0731.

*4.2.5. 1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3H-benz[e]indole (**15**)*

To a stirred mixture of **14** (30.1 g, 118 mmol) in dioxane (100 mL) at 0 °C was added trifluoroacetic anhydride (33.4 mL, 236 mmol), followed by Et_3N (49.2 mL, 354 mmol). The mixture was stirred at 0 °C for 10 min, at 20 °C for another 50 min, and then poured into ice-water (500 mL). Conc. HCl (40 mL) was added and the mixture was stirred at 20 °C for 20 min. The precipitated solid was filtered off, washed successively with dilute HCl (1N, 4 × 20 mL), water (10 × 50 mL), petroleum ether (5 × 25 mL), and dried to give **15** as a pale yellow solid (37.0 g, 100%) suitable for the following reactions; δ_H [$(CD_3)_2SO$] 8.32 (d, *J* = 9.0 Hz, 1 H), 8.07-7.97 (m, 3 H), 7.65-7.59 (m, 1 H), 7.56-7.51 (m, 1 H), 4.60-4.53 (m, 1 H), 4.50-4.40 (m, 2 H), 4.15 (dd, *J* = 11.3, 3.1 Hz, 1 H), 4.03 (dd, *J* = 11.3, 5.9 Hz, 1 H); consistent with that reported.[3]

*4.2.6. 1-(Chloromethyl)-3-(trifluoroacetyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonyl chloride (**16**)*

A solution of chlorosulfonic acid (1.77 mL, 26.5 mmol) in dry CH_2Cl_2 (20 mL) at 20 °C was added dropwise over 1 h to a stirred mixture of **15** (6.92 g, 22.1 mmol) and CH_2Cl_2 (60 mL) in an ice bath, maintaining the internal temperature at 3-4 °C throughout. The mixture was stirred for a further 2 h 30 min at the same temperature. Another batch of chlorosulfonic acid (1.77 mL, 26.5 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise over 45 min and the reaction was stirred in the ice bath for a further 1 h 30 min. At this point TLC (EtOAc:petroleum ether 1:4) indicated complete consumption of the starting material. Dry DMF (10 mL, sufficient to dissolve all suspended solid) was added, followed by oxalyl chloride (5.55 mL, 63.6 mmol). The reaction mixture was stirred in the ice bath for 30 min and then left to stand at 0-5 °C overnight. The solvent was evaporated under reduced pressure at 20 °C to give an amber solid. Cold water (100 mL) was added and the mixture was stirred at 0 °C (bath temperature) for 15 min. The solid was filtered off, washed with cold water (5 × 20 mL), and dried to give **16** as a pale yellow solid (8.8 g, 97%); mp 187-190 °C (lit. mp 189-192 °C[3]); δ_H ($CDCl_3$) 8.68-8.62 (m, 2 H), 8.10 (d, *J* = 9.0 Hz, 1 H), 8.09 (dd, *J* = 9.0, 2.0 Hz, 1 H), 8.00 (d, *J* = 9.0 Hz, 1 H), 4.68 (d, *J* = 11.6 Hz, 1 H), 4.50 (dd, *J* = 11.5, 8.6 Hz, 1 H), 4.30-4.24 (m, 1 H), 3.94 (dd, *J* = 11.6, 3.4 Hz, 1 H), 3.63 (dd, *J* = 11.6, 8.8 Hz, 1 H); consistent with that reported.[3]

*4.2.7. 1-(Chloromethyl)-5-nitro-3-(trifluoroacetyl)-1,2-dihydro-3H-benzo[e]indole-7-sulfonyl chloride (**7**)*

To a stirred solution of **16** (22.1 g, 53.6 mmol) in conc. H_2SO_4 (200 mL) at 0 °C was added solid KNO_3 (5.96 g, 59.0 mmol) portionwise over 2 h. After the addition was complete the mixture was stirred at 0 °C and reaction progress was monitored by TLC (EtOAc:petroleum ether 2:3). After 5 min it was found that significant starting material was still present, thus another batch of KNO_3 (1.19 g, 11.8 mmol) was added over a period of 20 min. After a further 5 min TLC analysis showed that the reaction was complete. The reaction mixture was poured onto ice (1.5 L) and the product was extracted into EtOAc (1.5 L). The EtOAc layer was washed with cold water, then dried ($MgSO_4$) and evaporated under reduced pressure at 30 °C to give a brown oil. To the oil was added CH_2Cl_2 (50 mL) and diisopropyl ether (100 mL), and the solution was stirred and then left at 5 °C overnight. The solid that formed was filtered off, washed with diisopropyl ether several times, and dried to give **7** (17.5 g, 71%) as a light brown solid. The mother liquor was left at −10 °C to give an additional crop (0.48 g, 2%); δ_H ($CDCl_3$) 9.35 (s, 1 H), 9.29 (d, *J* = 1.7 Hz, 1 H), 8.23 (dd, *J* = 9.0, 1.8 Hz, 1 H), 8.11 (d, *J* = 9.0 Hz, 1 H), 4.74 (d, *J* = 11.5 Hz, 1 H), 4.59 (dd, *J* = 11.4, 8.8 Hz, 1 H), 4.42-4.34 (m, 1 H), 3.95 (dd, *J* = 11.7, 3.5 Hz, 1 H), 3.74 (dd, *J* = 11.7, 7.7 Hz, 1 H); consistent with that reported.[3]

*4.2.8. Di(tert-butyl) 2-(1-(chloromethyl)-5-nitro-1,2-dihydro-3H-benzo[e]indole-7-sulfonamido)ethyl phosphate (**17**)*

A cold solution of **8** (1.98 g, 7.8 mmol) and *i*-Pr_2NEt (1.22 mL, 6.8 mmol) in THF (10 mL) was added to solution of **7** (2.98 g, 6.5 mmol) in THF (30 mL) at 0 °C. The mixture was stirred at 0 °C for a further 20 min, then solid Cs_2CO_3 (4.04 g, 12.4 mmol) and cold MeOH (20 mL) were added. The mixture was stirred at 0 °C for a further 10 min and then partitioned between EtOAc (400 mL) and ice-water (400 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc. The combined EtOAc extracts were washed again with water, then dried (Na_2SO_4), and evaporated under reduced pressure at 25 °C (bath temperature). The residue was dissolved in CH_2Cl_2 and the solution was filtered through a short silica column (Merck 230–400 mesh), eluting with CH_2Cl_2. The product-containing fractions were evaporated to give **17** as a red-orange foam (3.60 g, 95%); δ_H ($CDCl_3$) 8.90 (d, *J* = 1.7 Hz, 1 H), 7.90 (dd, *J* = 8.9, 1.8 Hz, 1 H), 7.78 (d, *J* = 8.9 Hz, 1 H), 7.72 (s, 1 H), 5.73 (t, *J* = 5.8 Hz, 1 H), 4.43 (s, 1 H), 4.14-3.94 (m, 5 H), 3.77 (dd, *J* = 11.2, 3.7 Hz, 1 H), 3.59 (dd, *J* = 11.0, 10.0 Hz, 1 H), 3.32-3.24 (m, 2 H), 1.46 (s, 9 H), 1.45 (s, 9 H).

*4.2.9. Di-tert-butyl 2-(1-(chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl phosphate (**18**)*

To a stirred mixture of **17** (2.91 g, 5.03 mmol) and **9** (2.06 g, 6.30 mmol) in DMA (80 mL) was added EDCI (1.94 g, 10.0 mmol) and anhydrous TsOH (175 mg, 1.00 mmol). The mixture was stirred at 20 °C for 2 h 30 min. Another batch of EDCI (1.94 g, 10.0 mmol) and anhydrous TsOH (88 mg, 0.50 mmol) were added and the mixture was stirred for a further 3 h 30 min. The reaction flask was cooled in an ice-bath and cold aqueous $NaHCO_3$ (5%, 160 mL) was added, followed by cold water (160 mL). After stirring at 0 °C for 5 min the precipitated solid was filtered off, washed with cold water several times, and dried to give **18** as a yellowish orange solid (4.06 g, 95 %); δ_H ($CDCl_3$) 9.41 (br s, 1 H), 9.33 (s, 1 H), 8.99 (d, *J* = 1.5 Hz, 1 H), 8.06 (dd, *J* = 8.9, 1.7 Hz, 1 H), 7.97 (d, *J* = 8.9 Hz, 1 H), 7.38 (d, *J* = 8.9 Hz, 1 H), 7.14 (d, *J* = 2.2 Hz, 1 H), 7.08-7.03 (m, 2 H), 6.11-6.03 (m, 1 H), 4.92 (dd, *J* = 10.8, 2.2 Hz, 1 H), 4.83 (apparent t, *J* = 9.8 Hz, 1 H), 4.39-4.32 (m, 1 H), 4.19 (t, *J* = 5.7 Hz, 2 H), 4.11-4.04 (m, 2 H), 3.97 (dd, *J* = 11.5, 3.4 Hz, 1 H), 3.80-3.74 (m, 4 H), 3.66 (dd, *J* = 11.5, 9.1 Hz, 1 H), 3.38-3.31 (m, 2 H), 2.86 (t, *J* = 5.7 Hz, 2 H), 2.66-2.58 (m, 4 H), 1.46 (s, 9 H), 1.44 (s, 9 H).

*4.2.10. 2-(1-(Chloromethyl)-3-(5-(2-morpholinoethoxy)-1H-indole-2-carbonyl)-5-nitro-2,3-dihydro-1H-benzo[e]indole-7-sulfonamido)ethyl dihydrogen phosphate trifluoroacetate (**10**)*

A filtered solution of **18** (3.78 g, 4.45 mmol) in CH_2Cl_2 (150 mL) was stirred with TFA (6.9 mL, 89 mmol) at 20 °C for 45 min. The mixture was concentrated under reduced pressure at 20 °C (bath temperature) to ca. 30 mL total volume. EtOAc (300 mL) was added and the mixture was stirred at 20 °C for 2 h 30 min. The resulting solid was filtered off, washed with EtOAc several times, and dried in vacuum over silica gel to give **10** as a yellowish orange solid (3.60 g, 95%); δ_H [$(CD_3)_2SO$] 11.84 (s, 1 H), ca. 10.7 (v br s, 1 H), 9.29 (s, 1 H), 8.87 (d, *J* = 1.7 Hz, 1 H), 8.45 (d, *J* = 8.9 Hz, 1 H), 8.27-8.20 (m, 1 H), 8.03 (dd, *J* = 8.9, 1.7 Hz, 1 H), 7.44 (d, *J* = 8.9 Hz, 1 H), 7.26 (d, *J* = 2.2 Hz, 1 H), 7.24 (d, *J* = 1.8 Hz, 1 H), 7.02 (dd, *J* = 8.9, 2.3 Hz, 1 H), 5.01-4.94 (m, 1 H), 4.71 (dd, *J* = 10.8, 2.1 Hz, 1 H), 4.68-4.62 (m, 1 H), 4.34-4.29 (m, 2 H), 4.19-4.09 (m, 2 H), 3.87-3.76 (m, 6 H), 3.26-3.13 (m, 4 H), 3.06-3.00 (m, 2 H), (2 H not observed, obscured by water peak δ 3.5-3.3); consistent with that reported.[6] Large scale batches (>1 g) made by this method had HPLC purity of 98.2-94.4%, as determined by the previously reported method.[6]

*4.2.11. Benzyl 2-((di(tert-butoxy)phosphoryl)oxy)ethylcarbamate (**20**)*

Using tetrazole: Tetrazole (3 wt% solution in acetonitrile, 468 mL, 159 mmol) was added gradually over 1 h to a stirred mixture of di-*tert*-butyl *N*,*N*-di-*iso*-propylphosphoramidite (95%, 53.0 mL, 159 mmol) and benzyl 2-hydroxyethylcarbamate (**19**) (26.0 g, 133 mmol) in THF (500 mL) at 20 °C under a nitrogen atmosphere. After the addition was complete the mixture was stirred at this temperature for 19 h. The mixture was cooled to 0 °C and H_2O_2 (30% aqueous, 48 mL, 494 mmol) was added. After 1.5 h at 0 °C the mixture was poured into ice-water and the product was extracted into EtOAc. The EtOAc layer was washed successively with cold aqueous Na_2CO_3 and water (× 2) and then dried (Na_2SO_4). The organic solvents were removed under

reduced pressure to give a colourless oil. A seed crystal was added and the mixture was allowed to stand at 20 °C for 1 h, then petroleum ether was added and the mixture was left at 5 °C for crystallisation to complete. The resulting solid was filtered off, washed with petroleum ether, and dried to give **20** (24.9 g, 49%) as a colourless solid; mp 43-45 °C; δ_H ($CDCl_3$) 7.37-7.28 (m, 5 H), 5.44 (br s, 1 H), 5.11 (s, 2 H), 4.07-4.00 (m, 2 H), 3.49-3.42 (m, 2 H), 1.47 (s, 18 H); consistent with that reported.[4] The mother liquor was left at 5 °C to give a second crop (12.9 g, 25%).[20]

Using imidazole and imidazole hydrochloride: To a stirred solution of **19** (1.07 g, 5.48 mmol) in DMF (20 mL) under nitrogen was added imidazole (0.75 g, 10.96 mmol) and imidazole hydrochloride (1.15 g, 10.96 mmol). Di-*tert*-butyl *N*,*N*-di-*iso*-propylphosphoramidite (95%, 2.73 mL, 8.20 mmol) was added dropwise and the resulting mixture was stirred at room temperature for 18 h. The mixture was cooled to 0 °C and H_2O_2 (30% aqueous, 1.17 mL, 14.96 mmol) was added dropwise. The mixture was stirred at 0 °C for 15 min, then allowed to warm to room temperature and stirred for a further 3 h. The mixture was cooled to 0 °C and aqueous Na_2SO_3 (10%, 25 mL) was added slowly. Water was added to dissolve the precipitated solids and the aqueous mixture was extracted with EtOAc (3 × 50 mL). The organic fractions were washed with water (100 mL), dried (Na_2SO_4), and the solvent was removed under reduced pressure. A seed crystal was added to the colourless oil and the mixture was allowed to crystallise at –4 °C overnight, giving **20** as a colourless solid (1.95 g, 92.0 %); ^{1}H NMR ($CDCl_3$) same as that described above.

*4.2.12. 2-Aminoethyl di(tert-butyl) phosphate (**8**)*

A solution of **20** (2.32 g, 5.99 mmol) in MeOH (100 mL) with Pd/C (5%, 0.46 g) was hydrogenated at 40 psi for 2.5 h. The mixture was filtered through Celite, washing with MeOH, and the filtrate was evaporated to give **8** (1.52 g, 100%) as a colourless oil; δ_H ($CDCl_3$) 4.01-3.94 (m, 2 H), 2.97-2.91 (m, 2 H), 1.53 (br s, 2 H), 1.50 (s, 18 H); consistent with that reported.[4]

*4.2.13. 4-(2-(4-Nitrophenoxy)ethyl)morpholine (**22**)*

To a stirred mixture of 4-nitrophenol (**21**) (42 g, 0.30 mol) and 4-(2-chloroethyl)morpholine hydrochloride (56 g, 0.30 mol) in anhydrous DMF (400 mL) was added dry K_2CO_3 (104 g, 0.75 mol). The mixture was stirred under nitrogen at 80-90 °C (bath temperature) for 3 h, then at 20 °C overnight (15 h). The mixture was poured into ice-water (1.5 L) and stirred at 0 °C for 1 h. The precipitated solid was filtered off, washed with water (6 × 100 mL) until the washes were colourless, then dried to give **22** as a pale yellow solid (68.4 g, 90%); mp 79-81 °C [lit. mp 82-83 °C (EtOAc/hexane)[21]]; δ_H ($CDCl_3$) 8.23-8.17 (m, 2 H), 6.99-6.94 (m, 2 H), 4.20 (t, *J* = 5.7 Hz, 2 H), 3.76-3.71 (m, 4 H), 2.84 (t, *J* = 5.7 Hz, 2 H), 2.62-2.55 (m, 4 H); consistent with that reported.[11] [Found: C, 57.36; H, 6.44; N, 11.25.$C_{12}H_{16}N_2O_4$ requires C, 57.13; H, 6.39; N, 11.10].

*4.2.14. 4-(2-Morpholinoethoxy)aniline (**23**)*

A solution of **22** (33.3 g, 132 mmol) in MeOH (170 mL) and THF (50 mL) with Pd/C (5%, ca. 3 g) was hydrogenated at 40 psi overnight (17 h). The mixture was filtered through Celite and the Celite plug was washed with CH_2Cl_2:MeOH (5:1) several times. The combined filtrates were evaporated to give **23** as a pink solid (29.0 g, 99%); δ_H ($CDCl_3$) 6.77-6.72 (m, 2 H), 6.65-6.60 (m, 2 H), 4.04 (t, *J* = 5.8 Hz, 2 H), 3.76-3.70 (m, 4 H), 3.41 (br s, 2 H), 2.76 (t, *J* = 5.8 Hz, 2 H), 2.60-2.54 (m, 4 H); consistent with that reported.[11] [Found: C, 64.94; H, 8.08; N, 12.66. $C_{12}H_{18}N_2O_2$ requires C, 64.84; H, 8.16; N, 12.60].

*4.2.15. Ethyl 5-(2-morpholinoethoxy)-1H-indole-2-carboxylate (**24**)*

A solution of sodium nitrite (9.95 g, 144 mmol) in water (27 mL) was added to a mixture of **23** (29.0 g, 131 mmol), water (223 mL) and conc. HCl (65.7 mL, 657 mmol) at 0 °C. The mixture was stirred for a further 10 min at 0 °C, then added to a stirred mixture of ethyl 2-methylacetoacetate (19.8 g, 138 mmol), sodium acetate (112 g, 1.37 mol), EtOH (163 mL), and ice (133 g, freshly added just before mixing) at 0 °C (bath temperature). The final reaction mixture was stirred at 0 °C for 5 min, then at 20 °C for 1 h 40 min. Solid Na_2CO_3 was added to give a pH of approximately 7-8. The mixture was extracted with CH_2Cl_2 (2 × 300 mL) and the extracts were washed with cold water (400 mL), then dried (Na_2SO_4) and evaporated to give a red oil. To the oil was added EtOH (60 mL) and HCl-saturated EtOH (100 mL). The mixture was stirred at reflux for 30 min, then cooled and evaporated under reduced pressure to give a dark solid. Water (200 mL) was added and the aqueous mixture was basified at 0 °C with 10% aqueous Na_2CO_3 solution to a pH of approximately 7-8. The resulting pale orange solid was filtered off, washed with water (5 × 80 mL), and dried to give **24** as a yellow solid (35.9 g, 86%); mp 130-132 °C; δ_H ($CDCl_3$) 8.78 (br s, 1 H), 7.30 (d, *J* = 8.9 Hz, 1 H), 7.13 (dd, *J* = 2.1, 0.8 Hz, 1 H), 7.08 (d, *J* = 2.4 Hz, 1 H), 7.01 (dd, *J* = 8.9, 2.4 Hz, 1 H), 4.40 (q, *J* = 7.1 Hz, 2 H), 4.15 (t, *J* = 5.8 Hz, 2 H), 3.77-3.71 (m, 4 H), 2.83 (t, *J* = 5.7 Hz, 2 H), 2.63-2.57 (m, 4 H), 1.41 (t, *J* = 7.1 Hz, 3 H); consistent with that reported.[11] [Found: C, 63.82; H, 7.23; N, 8.91. $C_{17}H_{22}N_2O_4$ requires C, 64.14; H, 6.97; N, 8.80].

*4.2.16. 5-(2-Morpholinoethoxy)-1H-indole-2-carboxylic acid hydrochloride (**9**)*

KOH (19.0 g, 339 mmol) was added to a mixture of ester **24** (35.9 g, 113 mmol), MeOH (250 mL) and water (125 mL) and the mixture was stirred at 20 °C for 1 h. The MeOH was evaporated under reduced pressure at 40 °C (bath temperature). The basic aqueous mixture was washed with ether (3 × 200 mL), filtered, and the filtrate was acidified with conc. HCl at 0 °C to pH 5-6. The precipitated solid was filtered off, washed with water many times (until the washings were no longer red), and dried in a vacuum oven at 50-60 °C for 7 h to give 5-(2-morpholinoethoxy)-1*H*-indole-2-carboxylic acid (31.3 g, 96%) as a pale yellow solid. Dioxane (300 mL) and HCl in dioxane (4M, 40.5 mL, 162 mmol) were added to this acid (31.3 g, 108 mmol) and the mixture was stirred at 20 °C for 15 h. The solid was filtered off, washed with EtOAc several times, and dried to give **9** as a grey-white solid (32 g, 91%); mp 251-254 °C; δ_H [$(CD_3)_2SO$] 12.87 (br s, 1 H), 11.65 (s, 1 H), 11.31 (br s, 1 H), 7.37 (d, *J* = 8.9 Hz, 1 H), 7.20 (d, *J* = 2.4 Hz, 1 H), 7.01 (dd, *J* = 2.0, 0.6 Hz, 1 H), 6.98 (dd, *J* = 8.9, 2.4 Hz, 1 H), 4.43 (t, *J* = 4.9 Hz, 2 H), 4.03-3.80 (m, 4 H), 3.60-3.45 (m, 4 H), 3.30-3.12 (m, 2 H); consistent with that reported.[11] [Found: C, 55.02; H, 5.89; N, 8.50. $C_{15}H_{18}N_2O_4$·HCl requires C, 55.13; H, 5.86; N, 8.57].

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6. Notes and references

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(20) ^{1}H NMR analysis showed that the second crop contained about 5% of the starting material **19**. However this second crop can still be used to obtain clean product in the subsequent reaction by proceeding as follows: the crude product from hydrogenolysis [of the second crop (11.7g)] was dissolved in CH_2Cl_2 and washed successively with aqueous Na_2CO_3 and water, and then dried (Na_2SO_4) and evaporated to give pure **8** (6.13 g, 80%) (clean by ^{1}H NMR analysis).

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7. Supplementary Material

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.tet.xxxx.xx.xxx. These data include ^{1}H NMR spectra for all compounds reported in this manuscript.