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Research Article

Synthesis of tritium labelled CHS 828 and its prodrug EB 1627

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Summary

The potent antitumoral cyanoguanidine CHS 828 and its prodrug EB 1627 have successfully been labelled with tritium. The pyridyl-3,5-dibromo derivative of CHS 828 was debrominated with tritium-gas using Pd/C catalyst to give [*pyridyl*-3,5-$^3H_2$]-CHS 828 with a radiochemical purity of 99.3% and a specific activity of 1960 GBq/mmol. Similarly, the pyridyl-3-bromo derivative of the prodrug EB 1627 was debrominated with tritium-gas to give [*pyridyl*-3-^{3}H]-EB 1627 with a radiochemical purity of 98.1% and a specific activity of 894 GBq/mmol. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: CHS 828; EB 1627; tritium; prodrug; tritio-debromination; Pd/C catalysed tritiation

Introduction

The cyanoguanidine CHS 828 (**1a**) has demonstrated potent antitumour activity *in vitro* and *in vivo*.[1,2] To overcome the variable absorption of CHS 828 in man[3] the soluble prodrug EB 1627[4,5] (**2a**) has been prepared. For biological studies[6] tritiated CHS 828 (**1d**) as well as its tritiated prodrug EB 1627 (**2d**) were needed. We decided to introduce tritium in the pyridine moiety of CHS 828 via tritio-debromination. A similar tritio-debromination of a substituted 3-bromopyridine has recently been reported.[7] Further, we wanted to introduce tritium in EB 1627 in the last synthetic step using the same method, but the outcome of this reaction would seem less predictable due to the labile polyethercarboxymethyl group (the prodrug moiety) attached to the

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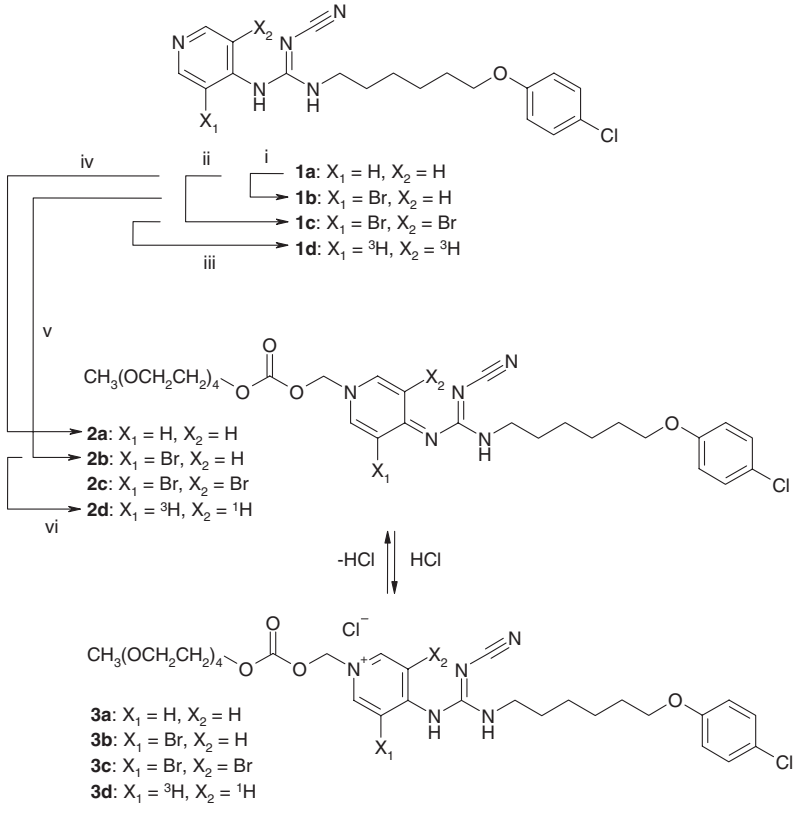
N-atom of the pyridine ring. We herein report on the labelling of CHS 828 and the prodrug EB 1627.

Results and discussion

CHS 828 (**1a**)[1] was brominated with one equivalent of N-bromosuccinimide (NBS) to give the mono-bromo compound **1b** (73% yield) and with an excess NBS to give the di-bromo compound **1c** (66% yield) (see Scheme 1). To obtain the highest specific activity of tritiated CHS 828, the di-bromo compound **1c** was used for tritio-debromination. Compound **1c** had low solubility in solvents commonly used for tritiations (methanol, ethanol and ethyl acetate) and impure reaction mixtures were obtained when hydro-debromination with H_2-gas and 5% Pd/C was attempted in those solvents. The best result was



Scheme 1. Synthesis of [$^3H_2$]-CHS828 and [^{3}H]-EB1627. Reagents and conditions: (i) NaH/DMF/NBS (1 eq)/0°C/1.5 h/73%; (ii) NaH/DMF/NBS (3.6 eq)/0°C/2.5 h/66%; (iii) $^3H_2$/Pd on C (5%)/diisopropylethylamine/THF/ room temp./24 h; (iv) See Peterson et al.[7] (v) Iodomethyl 2-(2-(2-(2-methoxy-ethoxy)-ethoxy)-ethoxy)-ethyl carbonate/CH_3CN/reflux/20 min/26%; (vi) $^3H_2$/Pd on C (10%)/EtOH/room temp./4 h

obtained when THF was used as solvent. Thus, compound **1c** was dissolved in THF and treated with tritium-gas, diisopropylethylamine and 5% Pd/C for 24 h. TLC purification afforded **1d** (1.08 GBq) with a radiochemical purity of 99.3% and a specific activity of 1960 GBq/mmol (average: 1.8 tritium per molecule). No tritio-dechlorination of the 4-chlorophenoxy ring was observed.

We decided to label the prodrug EB 1627 (**2a**) in the same position of the pyridine ring as in CHS 828 and using the same tritio-debromination procedure. In order to introduce tritium in the last step of the synthesis, attempts were made to synthesize **2b** from **1b** and **2c** from **1c** following the procedure for the preparation of **2a** from **1a**.[8] The best result was obtained in the alkylation of the mono-bromo compound **1b**, which gave **2b** in 26% yield. Two rotameric forms of this compound were observed by ^{1}H NMR. Prodrug EB 1627 (**2a**) and compound **2b** were soluble in most solvents, and ethanol with 10% Pd/C was used for the tritio-debromonation. Treatment with tritium-gas for 4 h followed by HPLC purification yielded **2d** (7.45 GBq) with a radiochemical purity of 98.1% and a specific activity of 894 GBq/mmol (0.84 tritium per molecule). Little or no degradation of the prodrug moiety was observed. Only after prolonged (overnight) treatment of **2b** with hydrogen-gas, reaction of the prodrug moiety was found. When THF was used as solvent, several reaction products were observed. The tritiated prodrug **2d** was isolated as the free base. The p*K* value for the equilibrium between the free base **2a** and its HCl salt **3a** was determined to be approximately 5.7. Chromatography of the salt **3a** in an unbuffered protic and neutral eluent (e.g. CH_2Cl_2/methanol 9:1) leads to separation of the free base **2a** from HCl.

Experimental

General

Preparative HPLC was performed using a Merck Hitachi apparatus with an L-4250 UV–VIS detector and an L-6000 pump. Columns used were Merck LiChrospher 250-10 containing either RP-18 (10 μm) or Si-60 (10 μm). Concentrations and specific activities were determined by HPLC by comparison of peak areas of radio-inactive reference compounds. A Packard Tri-Carb 2900TR Liquid Scintillation Analyzer was used to determine activity in liquid samples using Pico-Fluor^{TM} 40 (Packard) as scintillation cocktail. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX300 spectrometer. Chemical shifts are reported in ppm with tetramethylsilane (TMS, $\delta = 0.00$) as internal reference.

*N-[6-(4-Chlorophenoxy)hexyl]-N′-cyano-N″-4-(3-bromopyridyl)-guanidine (***1b***)*

Sodium hydride, (60% in oil, 0.48 g, 12 mmol) was added slowly to an ice-cooled stirred solution of CHS 828 (**1a**)[1] (3.7 g, 10 mmol) in DMF (25 ml).

After stirring for 15 min a solution of N-bromosuccinimide (1.8 g, 10 mmol) in DMF (10 ml) was added dropwise over 1 h. Stirring was continued for a further 15 min followed by addition of water (50 ml) and extraction with CH_2Cl_2 (3 × 50 ml). The organic extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was crystallized from CH_3OH to give (**1b**) in 73% yield (3.3 g). Mp. 151-2°C. ^{1}H NMR (DMSO-d_6) $\delta = 9.04$ (bs,1H), 8.68 (bs,1H), 8.43 (bs,1H), 7.76 (bt,1H), 7.31 (m,3H), 6.94 (d,2H), 3.95 (t,2H), 3.25 (q,2H), 1.71 (m,2H), 1.55 (m,2H), 1.38 (m,4H). ^{13}C NMR (DMSO-d_6) $\delta = 157.4$, 152.1, 151.1, 148.9, 129.1, 124.0, 116.1, 67.7, 41.5, 28.4, 28.3, 25.8, 25.0.

*N-[6-(4-Chlorophenoxy)hexyl]-N′-cyano-N″-4-(3,5-dibromopyridyl)-guanidine (***1c***)*

A stirred solution of CHS 828 (**1a**) (2.0 g, 5.4 mmol) in DMF (20 ml) was cooled in ice and treated slowly with sodium hydride, (60%, 0.26 g, 6.5 mmol) followed by N-bromosuccinimide (1.16 g, 6.5 mmol) 10 min later. The additions of sodium hydride and N-bromosuccinimide were repeated twice – after 75 and 150 min. The temperature was allowed to raise to room temperature, water (50 ml) was added and the mixture was extracted with CH_2Cl_2 (3 × 25 ml). The organic extracts were washed with saturated aqueous NaCl, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product was purified by chromatography on silica gel and elution with a CH_2Cl_2/CH_3OH gradient followed by crystallisation from CH_3OH to give (**1c**) in 66% yield (1.9 g). Mp. 156-7°C. ^{1}H NMR (DMSO-d_6) $\delta = 9.30$ (bs,1H), 8.79 (s,2H), 7.4 (br,1H), 7.31 (d,2H), 6.94 (d,2H), 3.95 (t,2H), 3.18 (br,2H), 1.70 (m,2H), 1.6–1.2 (m,6H). ^{13}C NMR (DMSO-d_6) $\delta = 157.4$, 151.2, 129.1, 123.9, 122.6, 116.6, 116.1, 67.6, 41.4, 28.7, 28.4, 25.7, 25.0.

*N-[6-(4-Chlorophenoxy)hexyl]-N′-cyano-N″-4-([3,5-$^3H_2$]pyridyl)-guanidine (***1d***)*

To a mixture of N-[6-(4-chlorophenoxy)hexyl]-N′-cyano-N″-4-(3,5-dibromo-pyridyl)-guanidine (**1c**) (10.2 mg, 0.019 mmol) and 5% Pd/C (12.4 mg) in tetrahydrofuran (1.0 ml) diisopropylethylamine (0.010 ml, 7.6 mg, 0.058 mmol) was added. The mixture was subjected to four freeze pump thaw cycles and the system was checked to be free of leaks. The reaction was stirred under a pressure of 1 bar of tritium gas at room temperature for 24 h. Thus a consumption of 230 GBq T_2 was calculated. After re-absorption of the tritium gas and evaporation of the solvent, the labile tritium was exchanged by adding 1 ml of methanol to the crude mixture and removing it *in vacuo*. This process was repeated three times. Finally the crude mixture was dried *in vacuo*. The product was extracted with 5 ml ethanol and the catalyst was removed by filtration. The activity of the colourless crude product was determined to be 42 GBq. Part of this solution (containing 1.85 GBq (50 mCi)) was worked up

(as described here): After removal of solvent *in vacuo* the residue was purified by preparative TLC (Merck, silica gel, 20 cm × 20 cm × 0.05 cm, eluent $CHCl_3$/EtOH/conc. NH_4OH 70/30/2 v/v/v) followed by a second TLC (Merck, RP-18, 20 cm × 20 cm, eluent: aq. $(NH_4)_2HPO_4$ (0.01 M) + $(n\text{-Bu})_4NHSO_4$ (0.01 M)/MeOH 20:80 v/v). The scraped-off TLC bands containing **1d** were placed on top of Dicalite® and eluted with ethanol to give an ethanolic solution of **1d**. The chemical and radiochemical purity was determined by HPLC on a Merck Hitachi apparatus (L-6200 pump) on a Lichrospher 125-4 RP-18 column with aq. $(NH_4)_2HPO_4$ (0.01 M) + $(n\text{-Bu})_4NHSO_4$ (0.01 M)/MeOH 35:65 v/v) as eluent (1 ml/min) and UV-detection (254 nm) (L-4250 UV–VIS detector) and radioactivity detection (Packard Flow System Analyzer Model D525F1 with Ultima-Flo™ M (Packard) as scintillation liquid). Radiochemical purity: 99.3%. Specific activity: 1960 GBq/mmol, and total activity: 1.08 GBq (58% isolated radiochemical yield from the crude reaction mixture).

*N-[6-(4-Chlorophenoxy)hexyl]-N′-cyano-N″-[3-bromo-(1-[2-(2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-carbonyloxymethyl)-1H-pyridin-4-ylidene-]-guanidine (***2b***)*

A solution of N-[6-(4-chlorophenoxy)hexyl]-N′-cyano-N″-4-(3-bromopyridyl)-guanidine (**1b**) (0.8 g, 1.8 mmol) in CH_3CN (30 ml) was heated to reflux and treated with a solution of iodomethyl 2-(2-(2-(2-methoxyethoxy)-ethoxy)-ethoxy)-ethyl carbonate[7] (1.1 g, 2.7 mmol) in CH_3CN (5 ml). After reflux for 20 min, the mixture was cooled to room temperature and evaporated *in vacuo*. The crude material was purified by chromatography on Sephadex LH-20 (150 g) with CH_2Cl_2/hexane (4:1) as eluent to provide 0.45 g of title compound which was further purified by chromatography on silica gel (25 g) with CH_2Cl_2/CH_3OH (95:5) as eluent to give **2b** as a pale yellow oil in 26% yield (0.33 g). The ^1H NMR spectrum shows two rotameric forms. Where the chemical shift of an assigned proton from the minor isomer is different from the major isomer, the signal from the minor isomer is given in brackets. ^1H NMR (DMSO-d_6) $\delta = 8.31$ [8.38] (d,1H), 7.89 [8.00] (t,1H), 7.71 [7.81] (dd,1H), 7.30 (d,2H), 6.95 (d,2H), 6.11 [6.38] (d,1H), 5.80 [5.82] (s,2H), 4.25 (m,2H), 3.95 [3.90] (t,2H), 3.62 (m,2H), 3.55–3.45 (m,10H), 3.42 (m,2H), 3.23 (s,3H), 3.14 [3.01] (q,2H), 1.71 (m,2H), 1.55–1.20 (m,6H). ^{13}C NMR (DMSO-d_6) $\delta = 168.8$, 157.4, 154.2, 153.4, 140.7, 139.0, 129.1, 123.9, 117.6, 116.1, 108.7, 107.5, 78.8, 71.2, 69.6, 69.5, 67.8, 67.6, 57.9, 41.3, 28.4, 28.3, 26.0, 25.1.

*N-[6-(4-Chlorophenoxy)hexyl]-N′-cyano-N″-[[3-^{3}H]-(1-[2-(2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-carbonyloxymethyl)-1H-pyridin-4-ylidene-]-guanidine (***2d***)*

A mixture of N-[6-(4-chlorophenoxy)hexyl]-N′-cyano-N″-[3-bromo-(1-[2-(2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy)-ethoxy]-carbonyloxymethyl)-1H-pyridin-

4-ylidene-]-guanidine (**2b**) (15.5 mg, 0.022 mmol) and Pd/C (10%, 3.1 mg) was suspended in ethanol (2.3 ml). The mixture was subjected to seven freeze pump thaw cycles and the system was checked to be free of leaks. The reaction was stirred under a pressure of 960 mbar of tritium gas at room temperature for 4 h. Thus a consumption of 204 GBq T_2 was calculated. After re-absorption of the tritium gas and evaporation of the solvent, the labile tritium was exchanged by adding 1 ml of methanol to the crude mixture and removing it *in vacuo*. This process was repeated three times. Finally the crude mixture was dried *in vacuo*. The product was extracted with 5 ml ethanol and the catalyst was removed by filtration. The activity of the crude product was determined to be 13.3 GBq. The filtrate was evaporated to dryness *in vacuo*. The final purification was achieved by preparative HPLC (detection at 254 nm) on an RP-18 column using CH_3CN:H_2O:HCOOH (50:50:1) as eluent followed by chromatography on an Si-60 column using CH_2Cl_2:MeOH (9:1) as eluent. The final product (**2d**) was dissolved and stored in ethanol solution. The chemical and radiochemical purity was determined by HPLC on a Merck Hitachi Lachrom 6000 apparatus (L-6200A pump) (column: YMC-Pack ODS-AQ, 3 μm, 150 × 4.6 mm, mobile phase: CH_3CN/AcOH-NaOAc buffer (0.03 M, pH 4.4) (1:1), 1 ml/min, UV-detection: 230 nm (Merck Hitachi UV Detector L-7400); radioactivity detection was performed on a Packard 500 TR Scintillation Analyzer with Ultima-Flo^{TM} M (Packard) as scintillation liquid). Radiochemical purity: 98.1%. Specific activity: 894 GBq/mmol, and total activity: 7.45 GBq (56% isolated radiochemical yield from the crude product).

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