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Research Article

Synthesis of deuterium-labelled chlorhexidine

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Abstract: The synthesis of deuterated chlorhexidine hydrochloride is reported. The specific labelling of the saturated chain is achieved through the combination of the previously reported synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol and a synthesis of carbon-14 labelled chlorhexidine. This synthesis begins with commercially available hex-2,4-yne-1,6-diol, its protection as a bis-tetrahydropyranylether, and exhaustive deuterogenation under rhodium catalysis to produce, after acid hydrolysis of the protecting groups, the saturated octadeuterated 1,6-hexanediol. Conversion of this material in two steps to the corresponding 1,6-diaminohexane was followed by the generation of guanidines with dicyanimide and final condensation with *p*-chloroaniline. The title compound was obtained in 10 chemical steps and in 6% overall yield. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: chlorhexidine; deuterium; synthesis

Introduction

Chlorhexidine is a common polydiguanide, known to possess both bacteriostatic and bactericidal properties.[1] Chlorhexidine was first synthesized by Rose and Swain[2] and through the last 50 years it has been used as a topical bactericide for skin infections, wounds, burns, in obstetrics and for bladder irrigation.[3] Even at high dilution chlorhexidine maintains a significant antibacterial activity.

Chlorhexidine is frequently used in dental hygiene.[4,5] Recently, mixtures of chlorhexidine with a variety of lipids has been reported to provide a superior antibacterial action, through a delayed release of the drug out of a drug–lipid formulation.[6] Entrapment of solvated chlorhexidine in lipid compartments (liposomes) has been proposed as the likely mechanism; however, solid state ^{2}H NMR spectra of deuterated lipid in chlorhexidine–lipid formulations do not support this morphology, suggesting instead that the chlorhexidine and lipid molecules form complexes, partitioning chlorhexidine away from the aqueous media.[7] To test the hypothesis of complex formation, the motional disorder of the saturated chain of the chorhexidine molecule needs to be examined and compared to the corresponding disorder present in the fatty acid chains of the phospholipids. A common way to measure the motional disorder is to examine the order parameters measured from the solid-state ^{2}H NMR spectra. This requires deuteration of chlorhexidine in the non-exchangeable proton positions, in the saturated chain portion of the molecule. In this manuscript, we report the preparation of octadeutero chlorhexidine (Figure 1).

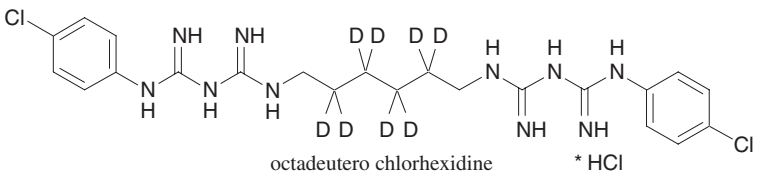

Figure 1 Structure of deuterium-labelled chlorhexidine.

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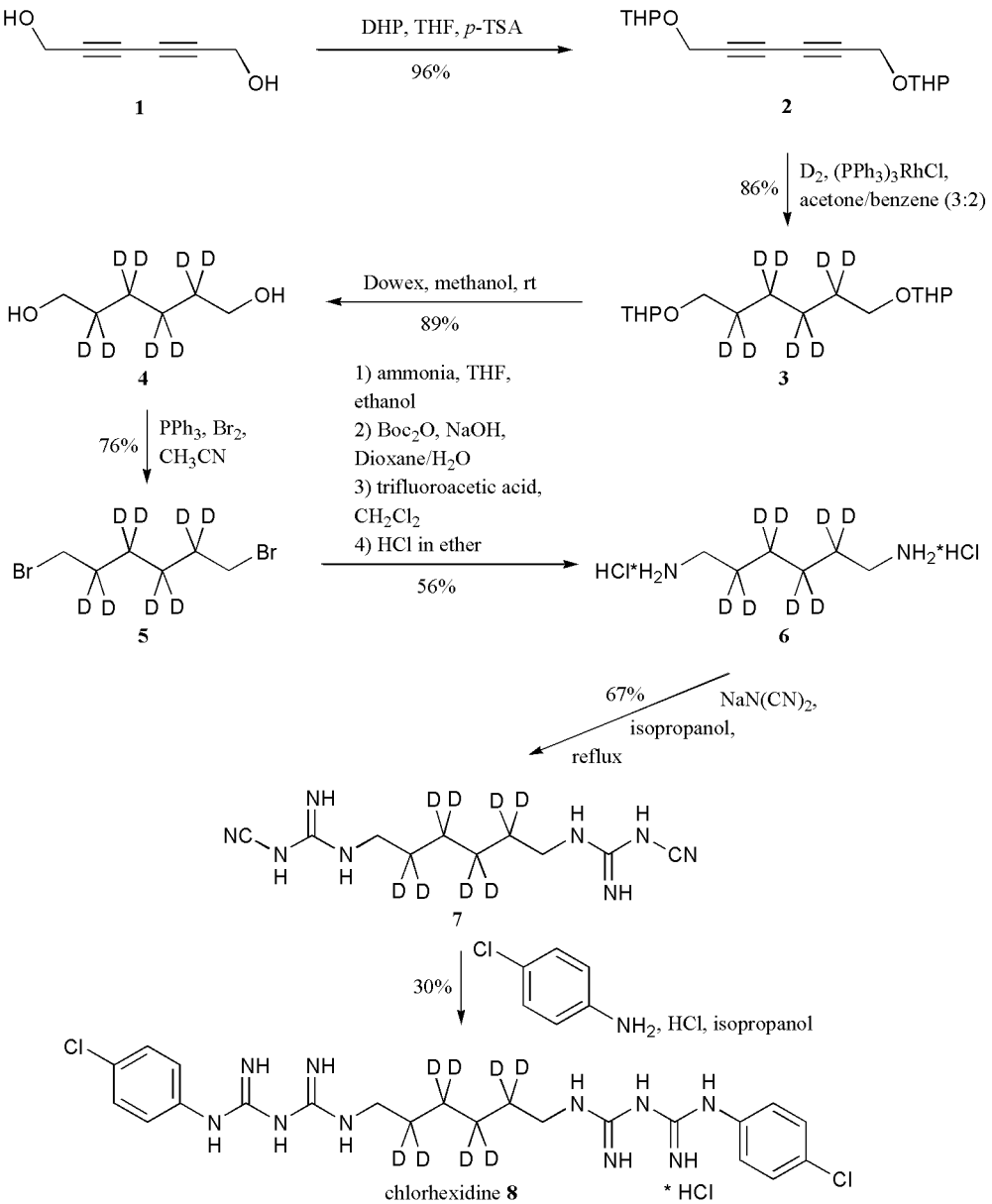

Scheme 1

Results and discussion

Chlorhexidine has been synthesized using a variety of pathways. Rose and Swain reported two different approaches. One is the reaction of *N*-(4-chlorophenyl)-cyanoguanidine in nitrobenzene at each of the amino groups of 1,6-hexamethylenediamine; alternatively, 1,6-di-(N^3-cyano-N^1-guanidino)hexane can be reacted with two equivalents of *p*-chloroaniline.[2] In the synthesis of Burns,[3] 1,4-dicyanobutane was obtained by treatment of 1,4-dibromobutane with potassium cyanide. 1,6-Diaminohexane was precipitated as the HCl–salt after reduction of the cyanogroups. Further conversion with dicyanimide and *p*-chloroaniline gave chlorhexidine. None of these three pathways could be used directly for the synthesis of ^{2}H-labelled analogue of chlorhexidine; deuterated *N*-(4-chlorophenyl)cyanoguanidine[2] or 1,4-dibromobutane[3] are not readily available. In addition, the Burns' pathway begins with a fully saturated protonated material, and deuterium substitution in saturated compounds is problematic. Finally, each of the three pathways involves multiple solvents and intermediate reagents that would also have to be replaced by their deuterated analogs, to prevent any possible exchange with their protons; thus an alternative pathway is required.

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The approach for the assembly of labelled version of chlorhexidine relies on a combination of the previously reported synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol[8] (**4**) and a synthesis of carbon-14 labelled chlorhexidine by Burns (Scheme 1).[3]

Commercially available hex-2,4-yne-1,6-diol (**1**) was protected in 96% yield to its bis-tetrahydropyranylether (**2**) and deuterated in acetone/benzene (3:2) with deuterium and Wilkensons' catalyst. After acid hydrolysis of the protecting groups 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol (**4**) was isolated in 77% over two steps. A 95% incorporation of deuterium into the 2,3,4,5-position of hexane-1,6-diol was ascertained from ^1H NMR spectrum, which was in accordance to the results reported in the literature for the synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol (**4**).[8]

Conversion of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol (**4**) into dibromide (**5**) using triphenylphosphine and bromine in acetonitrile and subsequent treatment with concentrated ammonia yield a diamine which was protected with *tert*-butylcarbamate allowing purification of the protected material by column chromatography on silica gel. The deuterated analogue of the Burns intermediate 1,6-diaminohexane dihydrochloride was obtained by cleavage of the protecting groups with trifluoroacetic acid and precipitation as dihydrochloride. 2,2,3,3,4,4,5,5-($^2H_8$)-1,6-Diaminohexane dihydrochloride (**6**) was further converted to deuterated chlorhexidine (**8**) accordingly to Burns procedure.[8] Therefore, the bis-guanidine was generated in 67% yield by treatment of 2,2,3,3,4,4,5,5-($^2H_8$)-1,6-diaminohexane dihydrochloride (**6**) with sodium dicyanimide in isopropanol under reflux. Final condensation of 6-[2,2,3,3,4,4,5,5-($^2H_8$)-hexamethylene]-bis-dicyandiamide (HMBDA) (**7**) with *p*-chloroaniline afford octadeuterated chlorhexidine hydrochloride (**8**) in 10 chemical steps and in 6% overall yield. A 95% incorporation of deuterium into the 2,3,4,5-position of hexane-1,6-diol moiety was again ascertained from ^1H NMR spectrum of chlorhexidine meaning no loss of isotopic purity was observed throughout the synthesis of chlorhexidine.

Experimentals

Materials

Tris(triphenylphosphine)-rhodium(I)chloride, sodium azide, sodium dicyanamide and *p*-chloraniline were purchased from Sigma–Aldrich, Oakville, Ontario, Canada and deuterium gas was provided by Praxair, Hamilton, Ontario, Canada. 2,4-Hexadiyne-1,6-diol was purchased from ScienceLab.com, Houston, Texas, and converted to 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diol as described in the literature.[8]

Synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-bis-1,6-(tetrahydropyran-2-yloxy)-hexane (3)[8]

To a clear, vigorously stirred pre-deuterated solution of 1.28 g tris(triphenylphosphine)-rhodium(I)chloride in 75 ml acetone/benzene (3:2, v/v) was added at room temperature under deuterium atmosphere in one portion bis-1,6-(tetrahydropyran-2-yloxy)-2,4-hexadiyne (5.05 g, 18.1 mol), dissolved in 12.5 ml of the same solvent mixture. After 20 h the solvents were distilled off, 25 ml of n-hexane was added and the resulting suspension was filtered through basic alumina (ca. 12.5 g). Elution with *n*-hexane, evaporation and drying *in vacuo* (1 h, 50°C, 0.1 mm) left 4.60 g pure **3** as colourless oil (15.6 mmol, 86%). A 95% incorporation of deuterium into the 2,3,4,5-position of the hexane-1,6-diol moiety was ascertained from ^1H NMR spectrum.

Synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-dibromide (5)

To a suspension of triphenylphosphine (4.90 g, 18.7 mmol) in 18.5 ml anhydrous acetonitrile cooled in an ice bath, bromine (2.92 g, 18.2 mmol) was added dropwise with stirring at such a rate that the mixture remains colourless. After addition was completed, the supernatant solution was slightly yellow. The ice bath was removed and a solution of diol (**4**) (1.15 g, 9.1 mmol) in 10 ml acetonitrile was added with stirring over a period of 15 min. The temperature of the mixture rose to 40–50°C and the precipitate dissolved completely for a short time. After the mixture cooled down to room temperature the solvent was removed via distillation. The residue was suspended in dry ethyl ether, filtered, and the collected solids were carefully washed five times with small portions of ether. The combined ether phases were concentrated and the residue distilled under reduced pressure (Kugelrohr at 130°C, 0.1 torr) to obtain 1.75 g dibromide (**5**) (6.9 mmol, 76%).

^1H NMR (300 MHz, $CDCl_3$) $\delta = 3.97$ (s, 4 H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.5 ppm.

Synthesis of 2,2,3,3,4,4,5,5-($^2H_8$)-hexane-1,6-diamine dihydrochloride (6)

To a solution of dibromide (**5**) (800 mg, 3.2 mmol) in 110 ml THF/ethanol (1:1) was added 55 ml concentrated ammonia and the reaction was stirred for 48 h. The solvent was removed under reduced pressure and the remaining solid was suspended in a solution of 0.55 g NaOH in 55 ml dioxane/H_2O (1:1). The

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suspension was treated with Boc_2O (3.01 g, 13.8 mmol) at 0°C for 1 h and then stirred at room temperature for further 3 h. The reaction mixture was extracted with CH_2Cl_2 (3×40 ml) and the combined extracts were evaporated under reduced pressure. The resulting mixture was purified by column chromatography on silica gel (hexane/ethyl acetate 7 : 3 + 0.1% NEt_3). The obtained product was dissolved in 20 ml CH_2Cl_2, 5 ml trifluoroacetic acid was added and the solution was stirred overnight. The solution was evaporated to dryness and 50 ml saturated HCl–ether solution was added. The solution was evaporated to dryness and the cycle was repeated a further two times. The product was obtained as white crystals in 56% yield (350 mg, 1.8 mmol).

^{1}H NMR (300 MHz, DMSO) $\delta = 7.90$ (br s, 6 H), 2.74 (s, 4 H) ppm; MS (Gly matrix): $m/z = 125$ ($M^+ - 2$ HCl).

Synthesis of 6-[2,2,3,3,4,4,5,5-($^2H_8$)-hexamethylene]-bis-dicyandiamide, (HMBDA) (7)

Diamine dihydrochloride (**6**) (306 mg, 1.6 mmol), sodium dicyanimide (306 mg, 3.4 mmol), and 7 ml over molecular sieves pre-dried isopropanol were stirred and refluxed for 16 h (bath temperature 90–100°C), and then heated for 20 h at 85°C. The white suspension was centrifuged to separate, and the product was washed with water (3×5 ml) before drying under reduced pressure at 45°C for 16 h, to give **7** as a white solid in 67% yield.

MS (FAB): $m/z = 259$ (M^++H).

Synthesis of deuterated chlorhexidine (8)

p-Chloroaniline (263 mg, 2.08 mmol) was stirred with isopropanol (1 ml). A solution of concentrated hydrochloric acid in isopropanol (192 mg in 1 ml) was added, and the mixture was stirred for 30 min to give a clear solution. HMBDA (**7**) (268 mg, 1.4 mmol) dissolved in isopropanol was added to the reaction mixture and then heated for 7 h to 85–90°C. After cooling the white gelatinous product was stirred at room temperature for 12 h. On addition of aqueous sodium hydroxide solution (2.7%, 3.3 ml) the white dihydrochloride dissolved and crude chlorhexidine was slowly depositing during 2 h as monohydrochloride. The product was separated by centrifugation and washed with isopropanol (2×0.7 ml), isopropanol/water (1:1) (2×0.7 ml) and water (4×0.7 ml) before being dried under reduced pressure at ambient temperature for 16 h. This gave 238 mg of a white solid, which was eight times recrystallized from freshly distilled ethanol to yield 30% of deuterated chlorhexidine hydrochloride (**8**) (162 mg, 0.32 mmol). A 95% incorporation of deuterium into the 2,3,4,5-position of the hexa-1,6-diamine moiety was ascertained from ^{1}H-NMR spectrum.

^{1}H-NMR (300 MHz, D_2O) $\delta = 7.27$ (br s, 4 H), 7.12 (br s, 4 H), 2.96 (s, 4 H) ppm; ^{13}C NMR (75 MHz, D_2O) δ 159.7, 156.6, 135.5, 130.4, 129.1, 124.9, 41.3 ppm; IR ($CHCl_3$) ν 3661, 3314, 1634, 1581, 1533, 1492, 1416, 1349, 1249, 1093, 824, 723 cm^{-1}; LC–MS (ESI-positive): $m/z = 549$ (M^++HCl), 513 (M^+).

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