

BISPHOSPHONIC COMPOUNDS. I. PREPARATION OF ^{13}C - AND ^{14}C -LABELLED CLODRONATE.

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SUMMARY

Tetrakis(1-methylethyl) (methylene- ^{13}C)bisphosphonate was obtained from ^{13}C -methyl iodide via the Michaelis-Arbuzov reaction with tris(1-methylethyl)phosphite followed by condensation with chlorophosphonic acid bis(1-methylethyl)ester. Tetrakis(1-methylethyl) (methylene- ^{14}C)bisphosphonate was prepared from ^{14}C -dibromomethane via the Michaelis-Arbuzov reaction with excess of tris(1-methylethyl)phosphite. Both of these labelled tetraesters were chlorinated with NaOCl, hydrolysed with HCl and neutralized with NaOH similarly. The overall yield of ^{13}C -clodronate, (dichloromethylene- ^{13}C)bisphosphonic acid disodium salt tetrahydrate, was 71 % with 99.5 % isotopic and 99.8 % chemical purity. The overall radiochemical yield of ^{14}C -clodronate was 9.8 %.

Key words: Synthesis, ^{13}C , ^{14}C , clodronate, ^{31}P NMR

INTRODUCTION

Clodronate, (dichloromethylene)bisphosphonic acid, disodium salt, tetrahydrate, is a powerful bone therapeutic agent for excess bone resorption¹. Analysis of clodronate on ng level using spectroscopic (UV, IR) methods cause severe problems, due to lack of proper chromophore groups. Mass spectrometry requires a suitable standard and for this purpose ^{13}C -clodronate was prepared. The ^{14}C -labelled clodronate was synthesized in order to investigate the behaviour of clodronate in biological systems²⁻⁵. In this paper we describe a convenient and inexpensive method to produce ^{13}C -clodronate on a multigram scale and a useful way to produce ^{14}C -clodronate.

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DISCUSSION

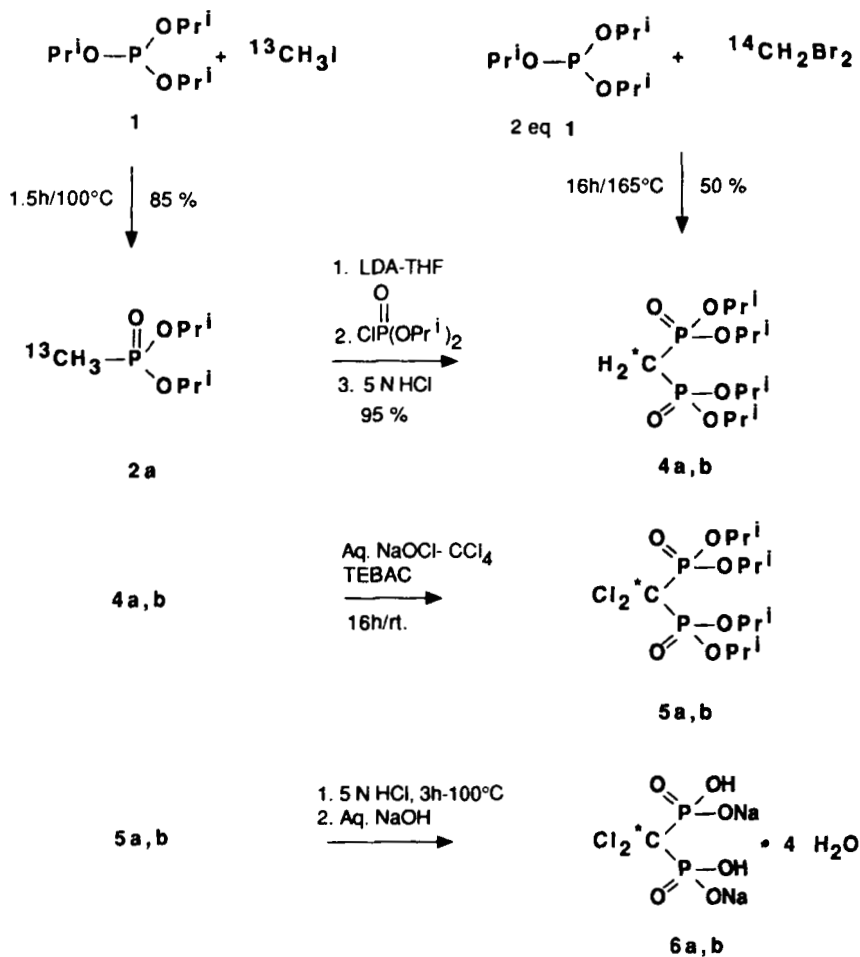
Quantification of clodronate at the ng level, by mass spectrometry, requires a suitable standard. Standards such as (dibromo- and bromochloromethylene)bisphosphonic acids proved inappropriate, because their chemical behaviour under mass spectrometric conditions was rather different to that of clodronate. One obvious solution is to isotopically label clodronate at oxygen, phosphorus, chlorine or carbon. Radioactive derivatives are not suitable for long term use. Cost and unambiguity of labelling are also important considerations. Although $^{17}\text{O}/^{18}\text{O}$ labelling would be advantageous, preparation of chemically stable oxygen labelled clodronate would be difficult. Hence the most appropriate labelling of clodronate is with carbon isotopes.

The commercial synthesis of clodronate is based on the Michaelis-Arbusov reaction between dibromomethane and tris(1-methylethyl)phosphite followed by chlorination, hydrolysis and neutralization.⁶ This method is also suitable for the preparation of small amounts of ^{14}C -clodronate, starting from ^{14}C -labelled dibromomethane. Although this route is also suitable for ^{13}C labelling, an alternative method commencing from ^{13}C -methyl iodide proved superior in terms of cost, purity and yield.

Scheme 1 shows the reaction sequences employed. The Michaelis-Arbusov reaction of **1** with $^{13}\text{CH}_3\text{I}$ gave phosphonate **2a**. In order to obtain optimum yields it is very important that both starting materials should be as pure as possible and that the temperature during addition must be carefully controlled. The key step in the synthesis is the satisfactory condensation of **2a** to **3** with lithium diisopropylamide (LDA), which requires absolutely dry conditions and freshly distilled materials.⁷ Due to this requirement for absolute dryness the butyllithium method is not recommended for small scale synthesis of ^{14}C -clodronate. The route to ^{14}C -labelled **4b** is principally the same as the commercial synthesis, combining two phosphite molecules with ^{14}C -dibromomethane. TLC purification with UV detection of **4b** was used to separate impurities from the tetraester.

The chlorination of **4** to **5** using sodium hypochlorite under phase transfer conditions was achieved by a modification of the method of McKenna.⁸ The synthesis of clodronate was completed as previously described.⁶ The pH adjustment with NaOH should be done in a well

cooled flask with vigorous stirring, because clodronate tends to decompose under alkaline conditions. The overall yield of ¹³C-clodronate was 71 % with 99.5 % isotopic and 99.8 % chemical purity. For ¹⁴C-clodronate, the overall chemical yield of clodronate was 41 %, but the total radiochemical yield was only 9.8 % possibly due to autoradiolysis of the starting ¹⁴C-dibromomethane.



Scheme 1. Preparation of ¹³C- and ¹⁴C-clodronate.

¹H, ¹³C and ³¹P NMR spectroscopy was used in verification of the products and their purity in the ¹³C-clodronate synthesis. Isotopic purity was determined from ³¹P NMR spectra

in which spin-spin coupling between ^{13}C and ^{31}P nuclei gives rise to a strong doublet in the phosphorus spectra, while the $\text{P-}^{12}\text{C-P}$ backbone gives a weak singlet between that doublet.

NMR spectra were recorded on a Bruker AC 250/Aspect 3000 spectrometer using a 10 mm multiprobe and radioactivity was measured using a LKB Wallac 1214 Rackbeta liquid scintillation counter. The identity of the radioactive compounds was established by comparison of TLC characteristics with those of authentic unlabelled samples. Unlabelled clodronate was identified from TLC using the Hahn-Luckhans reagent.

EXPERIMENTAL

^{13}C -Methyl iodide: Supplied by Aldrich-Chemie GmbH. ^{13}C -content 99.5%.

^{14}C -Dibromomethane: Supplied by Amersham. Specific activity 71 $\mu\text{Ci/mg}$.

Bis(1-methylethyl) (methyl- ^{13}C)phosphonate (2a): $^{13}\text{CH}_3\text{I}$ (10 g, 0.07 mol) was added to a dry three-necked flask. In to a dropping funnel was weighed tris(1-methylethyl)phosphite (14.6 g, 0.07 mol) under nitrogen. A portion of the phosphite (2 g) was added to the flask, with warming, until the reaction started. The rest was added dropwise, while keeping the mixture boiling, and heating under reflux was continued for 1 h after addition. Toluene (80 ml) was added to the cooled mixture followed by washing twice with 2 N NaOH (ca. 15 ml). The residue after drying (MgSO_4) and evaporation was distilled under reduced pressure (bp. 27-29°C/2mm). Chemical yield: 10.8 g, 85 %. Chemical purity 97 %. Isotopic purity 99.5 %. ^{31}P NMR (CDCl_3): δ 28.89 ppm, $^1J_{\text{CP}}=145.4$ Hz.

Chlorophosphonic acid bis(1-methylethyl)ester (3): See reference 9.

Tetrakis(1-methylethyl) (methylene- ^{13}C)bisphosphonate (4a): This reaction requires dry conditions. The apparatus used was dried for 2 h 120°C and cooled under dry nitrogen. Butyllithium (2.5 M in hexane, 44 ml, 0.11 mol) and bis(1-methylethyl)amine (11.7 g, 0.12 mol) were added to dry tetrahydrofuran (THF, 80 ml) at -20°C and the mixture stirred at this temperature for 15 min. The solution was cooled to -75°C and **2a** (10 g, 0.055 mol) in THF (15 ml) added at -74°C. The mixture was allowed to react at -65°C for 15 min followed by addition of **3** (11.1 g, 0.055 mol) in THF (15 ml) at -74°C. After 15 min the mixture was warmed to -20°C over 70 min. 5 N HCl was added to the vigorously stirred solution to pH 5-6,

at -50°C. THF was evaporated and water (75 ml) was added. The mixture was extracted three times with CHCl₃ (ca. 150 ml), and the combined CHCl₃ layers were washed with 10 % NaHCO₃ (80 ml) and saturated NaCl (80 ml) solutions. The residue after drying (MgSO₄) and evaporation was dissolved in ether and allowed to stand overnight, the solids filtered and the filtrate evaporated. Chemical yield: 18.3 g, 96 %. Chemical purity: 97 %. Isotopic purity: 99.5 %. ³¹P NMR (CDCl₃): δ 18.02 ppm, ¹J_{CP}=138.1 Hz.

Tetrakis(1-methylethyl) (dichloromethylene-¹³C)bisphosphonate (5a): **4** (10.3 g, 0.03 mol), CCl₄ (50 ml), benzyltriethylammonium chloride (TEBAC, 3 g) and 10 % NaOCl solution (100ml) were added to a flask in this order with efficient stirring. After 17 h the layers were separated and the water layer extracted with CCl₄ (20 ml). The combined CCl₄ layers were washed twice with water (ca. 30 ml), dried (MgSO₄) and evaporated. Chemical yield: 12.4 g, 100 %. Chemical purity: 100 %. Isotopic purity: 99.5 %. ³¹P NMR (CDCl₃): δ 7.26 ppm, ¹J_{CP}=155.0 Hz.

(Dichloromethylene-¹³C)bisphosphonic acid disodium salt, tetrahydrate (6a): **5a** (12.3 g, 0.03 mol) was added to a flask containing 37 % HCl (50 ml) and the mixture heated at 100°C for 3 h, and evaporated to dryness. The residue was dissolved in 2-propanol (20 ml) and evaporated, and this procedure repeated twice. The solid residue was dissolved in H₂O (15 ml) and 5 N NaOH (12 ml) was added at 10°C under efficient stirring. After 5 min the solution was heated to 70°C and EtOH (40 ml) was added slowly. The precipitate was collected by filtration, washed with ethanol and dried to constant weight. Chemical yield: 9.4 g, 87 %. Chemical purity: 99.8 %. Isotopic purity: 99.5 %. ³¹P NMR (D₂O): δ 9.56 ppm, ¹J_{CP}=129.3 Hz.

Tetrakis(1-methylethyl) (methylene-¹⁴C)bisphosphonate (4b): ¹⁴C-Dibromomethane (290 mg, 1.66 mmol, 20.6 mCi) and carrier CH₂Br₂ (580 mg, 3.32 mmol) were dissolved in freshly distilled tris(1-methylethyl)phosphite (2.50 g, 12.0 mmol) and the mixture heated at 165°C for 16 h. Excess phosphite was distilled off and the residue was purified by TLC (CH₂Cl₂:n-C₃H₇OH, 140:10.5 v/v, Kieselgel 60 F-254). Chemical yield: 858 mg, 50 %.

Tetrakis(1-methylethyl) (dichloromethylene-¹⁴C)bisphosphonate (5b): **5b** was prepared from **4b** (858 mg, 2.49 mmol) by the method previously described for the preparation of **5a**. Chemical yield: 934 mg, 91 %.

(Dichloromethylene- ^{14}C)bisphosphonic, acid disodium, salt tetrahydrate (**6b**): **6b** was prepared from **5b** (934 mg, 2.25 mmol) by the method previously described for the preparation of **6a**. The radiochemical purity was 24 % by TLC (system as earlier or 0.2 M acetate buffer pH 5:acetone:tetrahydrofuran, 13:4:3 v/v, Polygram CEL 300). Chemical yield: 735 mg, 90 %. The overall radiochemical yield from ^{14}C -dibromomethane was 9.8 % and total activity 2.02 mCi.

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