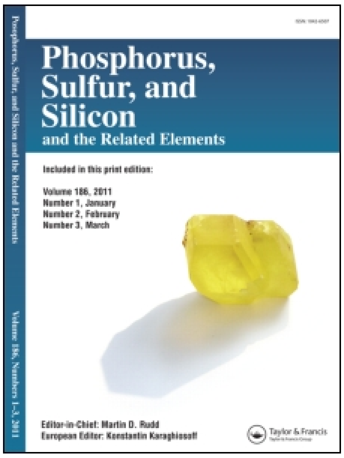

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p-SUBSTITUTED BENZYL HYDROXYBISPHOSPHONATES: SYNTHESIS AND HYDROLYSIS

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p-SUBSTITUTED BENZYL HYDROXYBISPHOSPHONATES: SYNTHESIS AND HYDROLYSIS

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Several hydroxybisphosphonate benzyl esters have been synthesized for study. The effect of the benzyl substituent on the acidic hydrolysis of the phosphonic esters has also been studied.

Keywords: Hydroxybisphosphonate; p-substituted benzyl esters; acidic hydrolysis

INTRODUCTION

Hydroxybisphosphonates are pyrophosphate analogs in which the oxygen bridge has been replaced by carbon, and diverse side chains have generated a large family of compounds. Hydroxybisphosphonates are becoming an important class of drugs used in the treatment of bone disease involving excessive bone resorption e. g. Paget's disease, post-menopausal osteoporosis[1]. Recently, different studies have shown that the use of bisphosphonates might be considered as an important improvement in the management of cancer[2]. It has been shown that bisphosphonates inhibit the development of bone metastasis in breast cancer patients[3]. Unfortunately, the molecular mode of action is not completely clear and may differ from compound to compound[4]. To gain a better understanding of the

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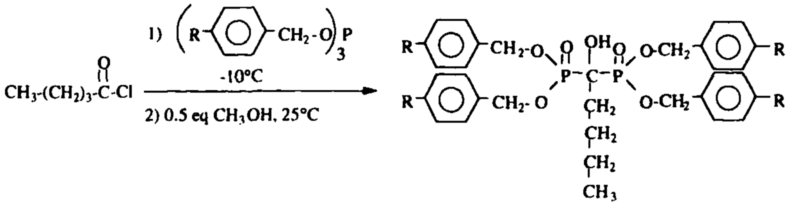
molecular mechanism of bones, our laboratory is working on the synthesis of bisphosphonate oligonucleotide conjugates. Oligonucleotide moiety could permit specific tissue targeting such as bone metastasis. In a previous study, our group detailed the first synthesis of oligonucleotide-bisphosphonate via a carbamate linker on the side chain of bisphosphonate[5]. This method has given good results in solution but not on solid phase. Our goal is thus to find a good coupling method which is appropriate to the oligonucleotide support polymer synthesis. The predominant method for oligonucleotide synthesis is the phosphoramidite approach[6]. The oligonucleotide chain extension is performed in three principal steps. Firstly, the deprotection of the 5′ end hydroxyl group of the CPG bound nucleoside. Secondly the coupling with the 3′ phosphoramidite with the next nucleoside in solution. Finally, the oxidation of the dinucleoside phosphite to the corresponding phosphate. The cycle starts again to obtain the desired oligonucleotide. At the end of the synthesis, aqueous ammonia is used to release the oligonucleotide from the polymer support and deprotect the exocyclic amino group of the nucleobases and the phosphate groups. Regarding this synthesis procedure, the covalent introduction of hydroxybisphosphonate to the oligonucleotide requires a phosphonate-protecting group easily cleavable and suited to the oligonucleotide. However, the use of a protecting group on the phosphonate moiety, removable in basic medium, is not possible here. It has been shown that the hydroxybisphosphonate tetraester rearranges itself in these conditions[7]. It is therefore necessary to find a protecting group which is releasable in weakly acidic conditions. In this study, we are reporting on the synthesis of benzyl esters and p-substituted benzyl esters of hydroxybisphosphonic acids and the study of hydrolysis conditions in an acidic medium. These derivatives are of interest because the same carboxylic esters are easily hydrolyzed in weak acidic media[8].

RESULTS AND DISCUSSION

Synthesis of p-substituted benzyl hydroxybisphosphonate esters

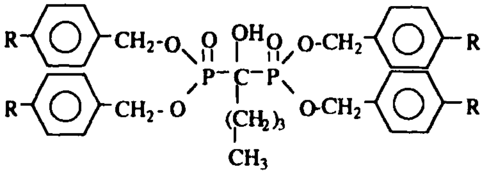
The classical strategy to synthesize hydroxybisphosphonic esters uses two steps. The first is an Arbusov reaction between an acid chloride and trialkylphosphite followed by an addition of dialkylphosphite on the corresponding α-ketophosphonate[9]. Unfortunately this procedure involves the preparation of trialkyl and dialkylphosphites. Recently, our group described an efficient one pot method to prepare hydroxybisphosphonic esters without the use of dialkylphosphite by introducing a protic reagent[10]. We decided to use this simple procedure to prepare p-substituted benzyl esters. We chose to use valeryl chloride which is added to benzyl phosphite derivatives prepared by nucleophilic substitution of p-substituted benzylic alcohol on tris(dimethylamino)phosphine, at a temperature of between −10°C and 0°C. The reaction is exothermic and the corresponding α-ketophosphonate is quickly generated. A protic reagent such as methanol is then added and the solution is stirred for one hour at ambient temperature (scheme 1).



SCHEME 1

As shown in Table I, this procedure allows us to synthesize several hydroxybisphosphonate benzyl esters with excellent yields except for compound 4 where R= OCH_3. When R= H; NO_2; CH_3, the reaction is very fast and leads in one hour at room temperature to a single product. When R = OCH_3, hydroxybisphosphonate is partially generated and the addition reaction stops itself. At the end of the reaction, the ^{31}P NMR spectrum shows a mixture of three products, hydroxybisphosphonate (19.20 ppm), α-ketophosphonate (-4.20 ppm) and dimethoxybenzylphosphite (6.10 ppm). The solution is then heated to 50°C to raise the yield but without success. On the other hand, a side reaction takes place which is consistent with the partial phosphonic esters hydrolysis of the α-ketophosphonate. This reaction is observed in $^{31}PNMR$ ($\delta = -5.0$ ppm).

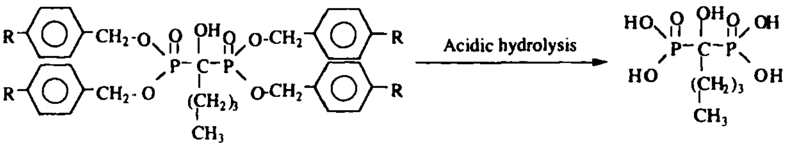
TABLE I



compound	*R*	*Yield %*	*$^{31}P\{^1H\}$ NMR δP(ppm)*	*^1H NMR*	
				δH (ppm)	*J (Hz)*
1	H	95	19.20	0.70 t (3H)	$^3J=7.5$
				1.15 se (2H)	$^3J=7.5$
				1.35–1.55m (2H)	
				1.90–2.10 m (2H)	
				5.10–5.30 m (8H)	
				7.20–7.40 m (20H)	
2	NO_2	85	19.40	0.80 t (3H)	$^3J=7.0$
				1.25–1.37 m (2H)	
				1.50–1.60 m (2H)	
				2.00–2.10 m (2H)	
				5.20–5.40 m (8H)	
				7.30 (8H)	$^2J=9.0$
				7.52 d (8H)	$^2J=9.0$
3	CH_3	85	19.20	0.93 t (3H)	$^3J=7.8$
				1.32–1.40 m (2H)	
				1.59–1.66 m (2H)	
				2.02–2.11 m (2H)	
				2.33 s (12H)	
				4.97–5.25 m (8H)	
				7.16 d (8H)	$^2J=8.0$
				7.25 d (8H)	$^2J=8.0$
4	OCH_3	35	19.30	0.93 t (3H)	$^3J=7.5$
				1.32–1.41 m (2H)	
				1.56–1.68 m (2H)	
				1.96–2.65 m (2H)	
				3.74 s (12H)	
				4.98–5.01 m (8H)	$^2J=8.0$
				6.81 d (8H)	$^2J=8.0$
				7.21 d (8H)	

Hydrolysis of p-substituted benzyl hydroxybisphosphonate esters

The usual method used to cleave benzyl esters is the hydrogenolysis reaction with the catalyst palladium on carbon[11]. In the case of hydroxybisphosphonate benzyl esters, catalytic hydrogenation does not allow us to obtain the corresponding acid. Different attempts have shown that the produced hydroxybisphosphonic acid is adsorbed on the surface of the catalyst. Acidic hydrolysis is therefore the preferred method. For the substituted benzyl carboxylic esters, several hydrolysis methods using weak acids such as trifluoroacetic acid or acetic acid are described in the literature[8]. For the corresponding phosphonic esters, two different acids are tested. The first one is trichloroacetic acid, which is completely compatible with the oligonucleotide. Indeed it is used to deprotect the hydroxyl group during the oligonucleotide synthesis. The second is chlorhydric acid, which is usually used for the acidic hydrolysis of a phosphonate group[12] (scheme 2).



SCHEME 2

For the chlorhydric acid hydrolysis, the influence of the acid concentration and the temperature has been studied. For the two methods, the reaction is monitored by ^{31}P NMR. The chemical shifts of hydroxybisphosphonate (19.20–19.40 ppm) and hydroxybisphosphonic acid (18.60 ppm) are quite different.

The results presented in Table II, show only the operating conditions involving the hydrolysis.

For each compound, trichloracetic acid is tested at 25 °C. For R = H; NO_2; CH_3, the ^{31}P NMR spectrum shows a single peak corresponding to the starting product. Raising the temperature and the duration time of the reaction do not improve the efficiency of the reaction. It seems that the presence of a strong electron-withdrawing group such as NO_2 or a weak electron-donating group such as CH_3, on the phenyl group has no effect on the rate of the reaction. On the other hand, the rate of hydrolysis is very fast when R = OCH_3. The reaction with trichloracetic acid is complete within a few minutes. For the compounds where R = H; NO_2; CH_3, the hydrolysis conditions were changed and trichloroacetic acid was replaced by chlorhydric acid. The results in table II show that the operating conditions are drastic (HCl 6N) and are therefore unsuitable for the oligonucleotide synthesis. However, the phosphonic benzyl esters are more easily hydrolysed than the phosphonic methyl esters. These last compounds are hydrolysed by using HCl 12N at reflux for 48 hours[12]. The phosphonic benzyl ester is less reactive than the p-substituted benzyl esters (R= NO_2, CH_3). For the non-substituted benzyl esters, it is necessary to heat to 50 °C to carry the reaction to completion.

TABLE II

R	*Experimental conditions of hydrolysis*	*Yield (%)*
H	HCl 6N; 50°C; 30 min	80
NO_2	HCl 6N; 25°C; 30 min	82
CH_3	HCl 6N; 25°C; 45 min	85
OCH_3	CCl_3COOH; 25°C, 1 min,	90

From the results of the present investigation, it can be concluded that to our knowledge the p-methoxybenzyl group is best for hydroxybisphosphonic acid synthesis. It can be removed with trichloracetic acid which is completely compatible with the oligonucleotide.

EXPERIMENTAL

Petroleum ether (99.95%, analytical reagent, PROLABO), $CHCl_3$ (HPLC grade, PROLABO) were dried by means of 4 Å molecular sieves. NMR experiments were performed on a VARIAN Unity Inova 500 spectrometer at 500.6 MHz for proton, 200.7 MHz for phosphorous and 125.9 MHz for carbon. The ^{31}P NMR and ^{13}C NMR spectra were recorded using phosphoric acid and methanol as external reference respectively. Tetramethylsilane and HOD were used as internal standards in $CDCl_3$ and D_2O for the ^1H NMR spectra.

General procedure for the synthesis of tribenzylphosphite $P(OCH_2\text{-}C_6H_4\text{-}pR)_3$

In a three necked flask equipped with a magnetic stirrer, a thermometer and a nitrogen inlet tube, one equivalent of tris (dimethylaminophosphine) and three equivalents of corresponding benzyl alcohol are heated to 100°C. The formed dimethylamine is swept out by a nitrogen gas current and is titrated progressively by acidimetry. The heating is stopped when 95% of the stoechiometric quantity has been titrated. The crude product is used without purification.

Tribenzylphosphite

Yield = 98%. Colorless oil.

^{31}P NMR ($CDCl_3$): δ: 137.80

^1H NMR ($CDCl_3$): δ: 7.2 (s, 15H, C_6H_5); 5.1 (d, 6H, $^3J_{HP}$=8.4 Hz, OCH_2)

Trinitrobenzylphosphite

Yield= 92%. Orange powder.

^{31}P NMR ($CDCl_3$): δ: 138.40

^1H NMR ($CDCl_3$): δ: 8.25 (d, 6H, $^2J_{HH}$=10 Hz, C_6H_4); 7.55 (d, 6H, $^2J_{HH}$=10 Hz, C_6H_4); 5.03 (d, 6H, $^3J_{HP}$=8 Hz, OCH_2)

Trimethylbenzylphosphite

Yield = 97%. White powder.

^{31}P NMR ($CDCl_3$):δ: 140.20

^1H NMR ($CDCl_3$): δ: 6.94 (d, 6H, $^2J_{HH}$=10 Hz, C_6H_4); 6.87 (d, 6H, $^2J_{HH}$=10 Hz, C_6H_4); 4.56 (d, 6H, $^3J_{HP}$=8.4 Hz, OCH_2); 2.08 (s, 9H, CH_3).

Trimethoxybenzylphosphite

Yield= 97%. White powder.

^{31}P NMR ($CDCl_3$): δ: 137.90

^1H NMR ($CDCl_3$): δ: 7.25 (d, 6H, $^2J_{HH}$=9 Hz, C_6H_4); 6.82 (d, 6H, $^2J_{HH}$=9 Hz, C_6H_4); 5.02 (d, 6H, $^3J_{HP}$=8.4 Hz, OCH_2); 3.80 (s, 9H, OCH_3).

General procedure for the synthesis of hydroxybisphosphonate benzyl esters

One equivalent of tribenzylphosphite in 2 ml of chloroform is placed in a three-necked flask equipped with a magnetic stirrer, an addition funnel, a thermometer and a nitrogen inlet tube. One equivalent of acid chloride in 2 ml of chloroform is slowly added to the stirred solution (exothermic reaction) at a temperature maintained between 0°C and −10°C. At the end of the addition, the solution is allowed to warm to room temperature. 0.5 eq. of methanol is added to the solution and ^{31}P NMR monitors the formation of hydroxybisphosphonate. After 1 hour, the solvent is evaporated under reduced pressure. The crude product is purified by washing twice with a mixture of ether /petroleum ether (1/1) to obtain a powder.

General procedure for the hydrolysis of hydroxybisphosphonate benzyl esters with trichloracetic acid

Hydroxybisphosphonate benzyl ester (1g) is placed in a three-necked flask equipped with a magnetic stirrer, an addition funnel, a thermometer and a nitrogen inlet tube. A solution of trichloroacetic acid in chloroform (2N) (25 ml) is then added. A white solid precipitate quickly forms in a few minutes. The solid is filtered under reduced pressure and washed with chloroform.

General procedure for the hydrolysis of hydroxybisphosphonate benzyl esters with chlorhydric acid

Hydroxybisphosphonate benzyl ester (1g) is placed in a three-necked flask equipped with a magnetic stirrer, an addition funnel, a thermometer and a nitrogen inlet tube. A solution of chlorhydric acid (25 ml) is then added. When the hydrolysis is complete (monitored by ^{31}P NMR), the aqueous solution is washed twice with chloroform (2x25 ml). The aqueous layer is then evaporated and lyophilized to leave a white powder.

^{31}P NMR (D_2O): δ: 18.60 ppm

^{1}H NMR (D_2O): δ: 0.80 (t, 3H, $^{3}J_{H\text{-}H}$ = 7.5 Hz, CH_3-CH_2); 1.25–137 (m, 2H, CH_3-CH_2); 1.50–1.60 (m, 2H, CH_3-CH_2-CH_2); 2.00–2.10 (m, 2H, -CH_2-CH_2-COH).

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