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The Synthesis of 1-Amino-2-Hydroxy- and 2-Amino-1-Hydroxy-Substituted Ethylene-1,1-Bisphosphonic Acids and their N-Methylated Derivatives

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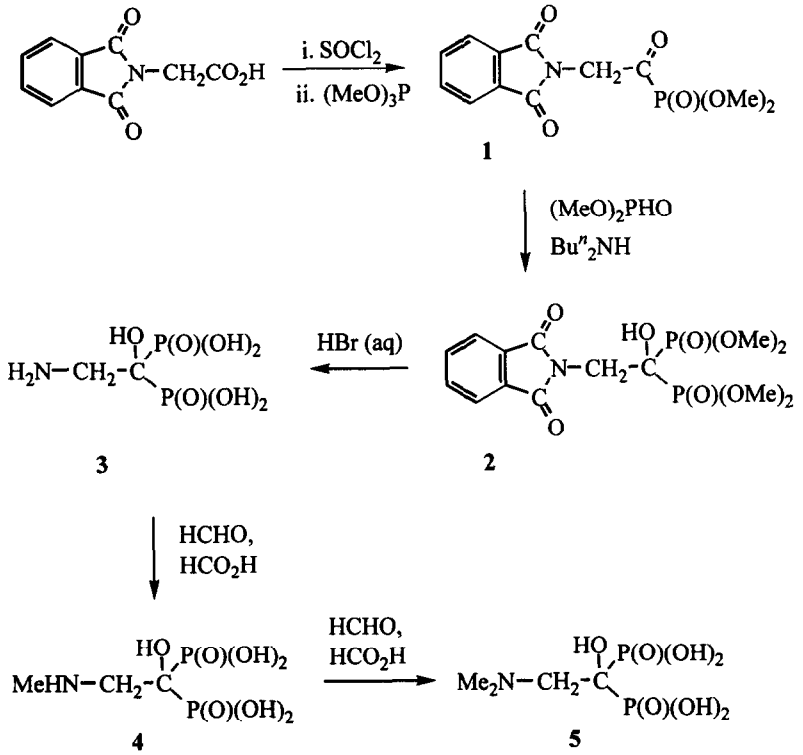
Abstract: A synthesis of 2-amino-1-hydroxyethylene-1,1-bisphosphonic acid **3** has been developed from *N*-phthaloylglycine *via* dimethyl 2-(*N*-phthaloylamino)acetylphosphonate **1**. The preparation of the *N*-methylated and *N,N*-dimethylated derivatives **4** and **5** has been achieved by the reaction of **3** with formic acid and formaldehyde. The synthesis of 1-amino-2-hydroxyethylene-1,1-bisphosphonic acid **9** (R=R'=H) and its *N*-methylated and *N,N*-dimethylated analogues has been achieved by the reaction of phosphorus trichloride and phosphorous acid with the appropriate *O*-benzyl protected hydroxyacetamide, followed by catalytic hydrogenolysis of the protecting group.

Bisphosphonates are of great interest in biology and medicine since they have a high affinity for mineralised tissue and can profoundly affect bone metabolism. We have been interested in these compounds in the context of developing improved ^{99m}Tc-based radiopharmaceutical imaging agents for bone scanning.[1] There are several agents which are currently used for bone scintigraphy including methylenebisphosphonic acid,[2] hydroxymethylenebisphosphonic acid,[3] and 1,1-diphosphono-2,3-dicarboxylic acid.[4] Nevertheless, it would be very desirable to improve on the performance of currently available agents by increasing target to non-target ratios and the speed with which these ratios can be achieved. One approach we have investigated involves the addition of polar substituents to an ethylene-1,1-bisphosphonic acid targeting unit. In this paper we report the synthesis of a series of mono-amino mono-hydroxy substituted systems of this type. In radiopharmaceutical application involving ^{99m}Tc the ligand is present in a large excess relative to the radionuclide during complex formation. For this reason, the ligands produced for this purpose must be of high purity to ensure that the radioactive complexes formed are derived from the ligands and not from impurities which may complex more effectively. Here we report suitable synthetic routes to a number of ethylene-1,1-bisphosphonic acids possessing amino and hydroxy substituents.

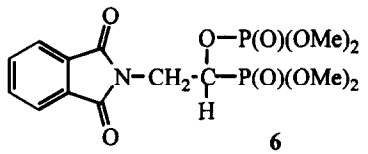
RESULTS AND DISCUSSION

Although 2-amino-1-hydroxyethylene-1,1-bisphosphonic acids have been prepared by the action of amines on the epoxide of ethylidene-1,1-bisphosphonic acid,[5] this route can also lead to the production of some of the corresponding 1-amino-2-hydroxyethylene-1,1-bisphosphonic acids. Furthermore, use of the epoxide of tetramethyl ethylidene-1,1-bisphosphonate leads to additional rearrangement products.[6] We have therefore approached the synthesis of the 2-amino systems **3**, **4**, and **5** from *N*-phthaloylglycine (Scheme 1).



Scheme 1

Treatment of *N*-phthaloylglycine with thionyl chloride gave the corresponding acid chloride which on reaction with trimethyl phosphite gave the corresponding α-ketophosphonate **1**. Addition of dimethyl phosphite across the α-carbonyl group in **1** to give the *N*-phthaloyl-protected 2-amino-1-hydroxyethylene-1,1-bisphosphonate **2** was successfully achieved in high yield by carrying out the reaction in toluene in the presence of di-*n*-butylamine as the catalyst. The choice of solvent was found to be important in this reaction since in chloroform, for example, the initially formed bisphosphonate **2** showed some tendency to rearrange to the corresponding phosphate **6**. Fortunately, it was found that the bisphosphonate **2** was only slightly soluble in toluene, so that in this solvent the product precipitated as it formed thus preventing subsequent rearrangement.



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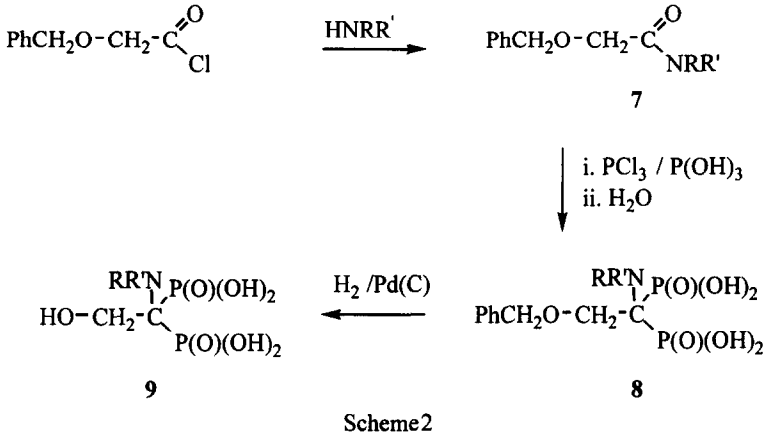
Hydrolysis of the bisphosphonate **2** with hydrobromic acid enabled the phthaloyl protecting group and the phosphonate esters to be removed in a single step to give the 2-amino-1-hydroxyethylene-1,1-bisphosphonic acid **3**.

By monitoring the reaction of this material with a mixture of formic acid and formaldehyde we were able to show that the *N*-methylation of this system occurred much more slowly than *N,N*-dimethylation, thus providing a very convenient route to 1-hydroxy-2-methylaminoethylene-1,1-bisphosphonic acid **4** and 2-dimethylamino-1-hydroxyethylene-1,1-bisphosphonic acid **5**.

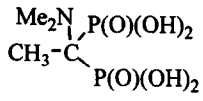


Scheme2

To synthesise the isomeric 1-amino-2-hydroxyethylene-1,1-bisphosphonic acids **9** we have extended an approach used for the preparation of a number of aminomethanebisphosphonic acids.[7] To introduce the 2-hydroxy group we used the *O*-benzyl protected hydroxyacetamides **7**, prepared by the action of the appropriate amine on benzyloxyacetyl chloride[8] (Scheme 2).

The reaction of these amides with phosphorus trichloride and phosphorous acid, followed by mild hydrolysis, gave the *O*-benzyl protected 1-amino-2-hydroxyethylene-1,1-bisphosphonic acids **8**. Catalytic hydrogenolysis of the benzyl protecting groups in **8** then gave the corresponding 1-amino-2-hydroxyethylene-1,1-bisphosphonic acids **9**. Interestingly, in the preparation of the *N,N*-dimethylated system **8** (R = R' = Me) a significant quantity of 1-dimethylaminoethylene-1,1-bisphosphonic acid **10** was also produced. Indeed, under certain conditions it became the major product, although the reasons for this are not immediately

obvious. In this particular case it was therefore necessary to purify the *O*-benzyl protected system **8** (R = R' = Me) by chromatography on cellulose.



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The hydrogenolysis of *O*-benzyl protected system **8** (R = R' = Me) also led to the formation of some 1-dimethylaminoethylene-1,1-bisphosphonic acid **10** and so once again chromatography on cellulose was needed to produce the pure ligand **9** (R = R' = Me).

Attempts to convert the amino-substituted system **9** (R = R' = H) to the corresponding *N*-methylated systems **9** (R = H, R' = Me) and **9** (R = R' = Me) using formic acid and formaldehyde have so far been unsuccessful.

The performance of the ^{99m}Tc complexes of these bisphosphonates as bone imaging agents has been evaluated and will be reported elsewhere.

EXPERIMENTAL

NMR spectra were obtained on JEOL FX100 and GSX270 spectrometers, chemical shifts in aqueous solution are with respect to an internal reference of 1,4-dioxan (δ_H 3.53; δ_C 66.5).

Dimethyl N-Phthaloylaminoacetylphosphonate **1**

N-Phthaloylglycine (10.25 g) was heated under reflux with thionyl chloride (15 cm^3) for 1.5 h. Toluene (20 cm^3) was then added and the toluene and excess thionyl chloride then removed under reduced pressure (55 °C at 15 mmHg). To a solution of the residue in toluene (10 cm^3), cooled in an ice bath, was then added slowly a solution of trimethyl phosphite (6.3 g) in toluene (10 cm^3). Additional trimethyl phosphite was added if necessary until ^{31}P NMR. spectroscopy indicated that the α-ketophosphonate **1** [δ_P(PhMe) -1.3] had formed and that the trimethyl phosphite was in slight excess. The reaction mixture was filtered and the solvent and volatile components removed under reduced pressure (50 °C at 0.01 mmHg) to give the ketophosphonate (**1**) (14 g, 95%) as a pale yellow viscous oil which crystallised on standing, M^+ 297 (Found: C, 48.64; H, 4.28; N, 5.00. $C_{12}H_{12}NO_6P$ requires C, 48.49; H, 4.07; N, 4.71%) $\delta_P(CDCl_3)$ -1.7; $\delta_H(CDCl_3)$ 3.92 (6H, d, J_{PH} 11 Hz, POMe), 4.90 (2H, d, J_{PH} 3 Hz, CH_2), 7.74 (2H, m, Ar-H), 7.84 (2H, m, Ar-H); $\delta_C(CDCl_3)$ 47.4 (d, J_{PC} 69 Hz, C-2), 53.9 (d, J_{PC} 7 Hz, POMe), 123.0 (C-3', C-6'), 131.3 (C-1', C-2'), 133.9 (C-4', C-5'), 166.5 (C=O), 202.8 (d, J_{PC} 175 Hz, C=O).

Tetramethyl 1-Hydroxy-2-(N-phthaloylamino)ethylene-1,1-bisphosphonate **2**

To a cooled solution of the α-ketophosphonate **1** (13 g) in toluene (40 cm^3) was added dimethyl phosphite (5.5 g) and di-*n*-butylamine (0.2 g) and the reaction left to stir overnight. After about 10 h the reaction mixture was filtered to give a white solid (10 g). The filtrate was left to stir at room temperature and over the following few days a further quantity of product precipitated from the reaction. The combined product was washed with toluene and then dried under reduced pressure. Tetramethyl 1-hydroxy-2-(*N*-phthaloylamino)ethylene-1,1-bisphosphonate (15 g, 84%) was obtained as a white solid, m.p. 148 °C, (Found: C, 41.57; H, 4.75; N, 3.51. $C_{14}H_{19}NO_9P_2$ requires C, 41.29; H, 4.70; N, 3.44%), $\delta_P(CDCl_3)$ 18.6; $\delta_H(CDCl_3)$ 3.86 (6H, d, J_{PH} 11 Hz, POMe), 3.90 (6H, d, J_{PH} 11 Hz, POMe), 4.40 (2H, m, CH_2), 5.65 (1H, t, J_{PH} 11 Hz, OH), 7.78 (2H, m, Ar-H), 7.90 (2H, m, Ar-H); $\delta_C(CDCl_3)$ 43.0 (br s, C-2), 54.3 (t, J_{PC} 3.5 Hz, POMe), 54.6 (t, J_{PC} 3.5 Hz, POMe), 75.9 (t, J_{PC} 157 Hz, C-1), 123.6 (C-3', C-6'), 131.5 (C-1', C-2'), 134.3 (C-4', C-5'), 169.0 (C=O).

2-Amino-1-hydroxyethylene-1,1-bisphosphonic acid **3**

Tetramethyl 1-hydroxy-2-(*N*-phthaloylamino)ethylene-1,1-bisphosphonate (8.14 g) was dissolved in hydrobromic acid (50 cm^3, 48%) and heated under reflux overnight. After cooling, the solution was evaporated to dryness under reduced pressure and the phthalic acid present taken up in hot ethanol. The bisphosphonate residue was then filtered off and dried. 2-Amino-1-hydroxyethylene-1,1-bisphosphonic acid **3** (3.7 g, 83%) was obtained as a white solid, m.p. 235—236 °C, (Found: C, 10.78; H, 3.78; N, 6.27. $C_2H_9NO_7P_2$ requires C, 10.87; H, 4.10; N, 6.34%); δ_P(HCl, 5M) 15.4; δ_H(HCl, 5M) 3.36 (2H, t, J_{PH} 12 Hz, 2-H); $\delta_P(D_2O$; pH 9) 13.4; $\delta_C(D_2O$; pH 9) 42.8 (C-2), 70.3 (t, J_{PC} 127 Hz, C-1).

1-Hydroxy-2-methylaminoethylene-1,1-bisphosphonic acid **4**

2-Amino-1-hydroxyethylene-1,1-bisphosphonic acid (0.25 g) was heated under reflux with a mixture of formaldehyde (2.5 cm^3, 37%) and formic acid (12.5 cm^3, 96%). After about 4 h the solid had dissolved. The mixture was then cooled and evaporated to dryness under reduced pressure. The residue was purified by repeatedly precipitating it from aqueous solution using ethanol. The N-methylated product **4** was dried under reduced pressure and isolated in good yield as a colourless solid, m.p. 138—139 °C, (Found: C, 15.29; H, 4.68; N, 5.76. $C_3H_{11}NO_7P_2$ requires C, 15.33; H, 4.72; N, 5.96%); δ_P(HCl, 5M) 15.0; δ_H(HCl, 5M) 2.65 (3H, s, Me), 3.39 (2H, t, J_{PH} 12 Hz, 2-H); δ_C(HCl, 5M) 34.3 (NMe), 51.5(C-2), 70.2 (t, J_{PC} 142 Hz, C-1).

2-Dimethylamino-1-hydroxyethylene-1,1-bisphosphonic acid **5**

2-Amino-1-hydroxyethylene-1,1-bisphosphonic acid (0.4 g) was dissolved in a mixture of formaldehyde (2.5 cm^3, 37%) and formic acid (12.5 cm^3, 96%), and the resulting solution heated under reflux for 3 days. The mixture was then cooled and filtered and the filtrate evaporated to dryness under reduced pressure. The residue was purified by repeatedly precipitating it from aqueous solution using ethanol. The pure N,N-dimethylated product **5** precipitate was dried under reduced pressure and isolated in high yield as a colourless solid, m.p. 213—215 °C, (Found: C, 19.28; H, 5.44; N, 5.74. $C_4H_{13}NO_7P_2$ requires C, 19.29; H, 5.26; N,

5.62%); δ_P(HCl, 5M) 14.8; δ_H(HCl, 5M) 2.85 (3H, s, Me), 3.49 (2H, t, J_{PH} 12 Hz, 2-H); δ_C(HCl, 5M) 47.0 (Me), 60.6(C-2), 70.9 (t, J_{PC} 143 Hz, C-1).

1-Amino-2-hydroxyethylene-1,1-bisphosphonic acid **9** (R = R' = H)

Benzyloxyacetamide[8] (23.4 g) was added to an initially stirred mixture of phosphorus trichloride (58.4 g) and phosphorous acid (11.6 g) and the mixture heated at 70 °C for 1 h. After cooling the excess phosphorus trichloride was decanted off and the residue hydrolysed by the careful addition of water (100 cm^3). This mixture was left to stir overnight, filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was then dissolved in the minimum quantity of water and the 1-amino-2-benzyloxyethylene-1,1-bisphosphonic acid **8** (R = R' = H) precipitated by the addition of acetone. This process was repeated until a pure product was obtained (17.5 g, 40%). δ_P(D_2O, pH ~14) 18.1; δ_H(D_2O, pH ~14) 3.67 (2H, t, J_{PH} 11 Hz, 2-H), 4.36 (2H, s, CH_2), 7.19—7.33 (5H, m, Ar-H); δ_C(D_2O, pH ~14) 57.8 (t, J_{PC} 126 Hz, C-1), 73.4 (br s, C-2), 76.6 (CH_2O), 128.0 (C-4'), 128.6 (C-3', C-5'), 128.8 (C-2', C-6'), 138.5 (C-1').

The hydrogenolysis of a portion of the 1-amino-2-benzyloxyethylene-1,1-bisphosphonic acid (8 g) was carried out at atmospheric pressure in aqueous solution using palladium on charcoal as the catalyst. The 1-amino-2-hydroxyethylene-1,1-bisphosphonic acid was purified by precipitating it from aqueous solution using acetone. The purified product **9** (R = R' = H) was obtained as a colourless solid (5.4 g, 95%), m.p. 180.5 °C, (Found: C, 10.98; H, 4.11; N, 6.38. $C_2H_9NO_7P_2$ requires C, 10.87; H, 4.10; N, 6.34%); δ_P(HCl, 5M) 11.1; δ_H(HCl, 5M) 3.93 (2H, t, J_{PH} 11 Hz, 2-H); δ_C(HCl, 5M) 57.7 (t, J_{PC} 135 Hz, C-1), 60.7 (s, C-2); δ_P(D_2O, pH 12) 17.0; δ_H(D_2O, pH 12) 3.58 (2H, dd, J_{PH} 10 and 12 Hz, 2-H); δ_C(D_2O, pH 12) 58.5 (t, J_{PC} 120 Hz, C-1), 63.8 (br s, C-2).

2-Hydroxy-1-methylaminoethylene-1,1-bisphosphonic acid **9** (R = H, R' = Me)

N-Methyl benzyloxyacetamide[9] (27 g), prepared by the action of methylamine on benzyloxyacetyl chloride,[8] was slowly added to an initially stirred mixture of phosphorus trichloride (62 g) and phosphorous acid (12.4 g) and the mixture heated at 70 °C for 1.5 h. After cooling, the excess phosphorus trichloride was decanted off and the residue hydrolysed by the careful addition of water (200 cm^3). This mixture was left to stir for 2 h, filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was then dissolved in the minimum quantity of water and the 2-benzyloxy-1-methylaminoethylene-1,1-bisphosphonic acid **8** (R = H, R' = Me) precipitated by the addition of acetone. This process was repeated until a pure product was obtained (18.6 g, 39%). δ_P(D_2O; pH <1) 12.4; δ_P(D_2O; pH ~14) 15.7; δ_H(D_2O; pH ~12) 2.15 (3H, s, NMe), 3.72 (2H, t, J_{PH} 11 Hz, 2-H), 4.36 (2H, s, CH_2), 7.17—7.32 (5H, m, Ar-H); δ_C(D_2O; pH ~12) 29.8 (t, J_{PC} 6 Hz, NMe), 62.9 (t, J_{PC} 125 Hz, C-1), 70.2 (br s, C-2), 73.2 (s, CH_2), 128.0 (C-4'), 128.6 (C-3', C-5'), 128.7 (C-2', C-6'), 138.4 (C-1').

The hydrogenolysis of 2-benzyloxy-1-methylaminoethylene-1,1-bisphosphonic acid (18 g) was carried out in aqueous solution at atmospheric pressure using palladium on charcoal as the catalyst. The 2-hydroxy-1-methylaminoethylene-1,1-bisphosphonic acid was purified by precipitating it from aqueous solution using

acetone. The purified product **9** (R = H, R' = Me) was obtained as a white solid (10.2 g, 79%), m.p. 156—158 °C, (Found: C, 15.58; H, 5.0; N, 5.8. $C_3H_{11}NO_7P_2$ requires C, 15.33; H, 4.72; N, 5.96%); δ_P(HCl, 5M) 10.2; δ_H(HCl, 5M) 2.73 (3H, s, NMe), 3.99 (2H, t, J_{PH} 11 Hz, 2-H); δ_C(HCl, 5M) 30.6 (NMe), 59.0 (C-2), 63.1 (t, J_{PC} 133 Hz, C-1).

1-Dimethylamino-2-hydroxyethylene-1,1-bisphosphonicacid **9** (R = R' = Me)

N,N-Dimethyl benzyloxyacetamide (35 g), prepared by the action of dimethylamine on benzyloxyacetyl chloride,[8] was slowly added to an initially stirred mixture of phosphorus trichloride (76 g) and phosphorous acid (15.2 g) and the mixture heated at 70 °C for 2 h. After cooling the excess phosphorus trichloride was decanted off and the residue hydrolysed by the careful addition of water (150 cm^3). This mixture was left to stir for 2 h, filtered, and the filtrate evaporated to dryness under reduced pressure. The residue was then dissolved in the minimum quantity of water and the product precipitated by the addition of acetone. NMR spectroscopy showed this material to be a mixture of 2-benzyloxy-1-dimethylaminoethylene-1,1-bisphosphonic acid **8** (R = R' = Me) and 1-dimethylaminoethylene-1,1-bisphosphonic acid **10** together with some phosphorous acid. The pure 2-benzyloxy-1-dimethylaminoethylene-1,1-bisphosphonic acid was isolated from this mixture by chromatography on cellulose (Merck microcrystalline cellulose - Avicel®) using methanol as the eluant. δ_P(HCl, 5M) 9.1; δ_H(HCl, 5M) 2.86 (6H, s, NMe_2), 3.85 (2H, br dd, J_{PH} 9 and 11 Hz, 2-H), 4.45 (2H, s, OCH_2), 7.15—7.29 (5H, m, Ar); δ_C(HCl, 5M) 42.5 (NMe_2), 66.4 (br s, C-2), 68.9 (t, J_{PC} 130 Hz, C-1), 73.4 (OCH_2), 128.6 (C-4'), 128.7 (x2)(C-3', C-5'), 128.9 (x2)(C-2', C-6'), 136.1 (C-1'); δ_P(pH ~14) 12.6; δ_H(pH ~14) 2.65 (6H, s, NMe_2), 3.87 (2H, t, J_{PH} 9 Hz, 2-H), 4.36 (2H, s, OCH_2), 7.19—7.33 (5H, m, Ar); δ_C(pH ~14) 41.5 (NMe_2), 70.0 (t, J_{PC} 115 Hz, C-1), 71.3 (br s, C-2), 73.0 (OCH_2), 128.2 (C-4'), 128.7 (x2)(C-3', C-5'), 128.9 (x2)(C-2', C-6'), 137.7 (C-1').

The hydrogenolysis of 2-benzyloxy-1-dimethylaminoethylene-1,1-bisphosphonic acid was carried out at atmospheric pressure in aqueous solution, using palladium on charcoal as the catalyst, to give 2-hydroxy-1-dimethylaminoethylene-1,1-bisphosphonic acid together with some 1-dimethylaminoethylene-1,1-bisphosphonic acid. A pure sample of the 2-hydroxy-1-dimethylaminoethylene-1,1-bisphosphonic acid (2 g) was isolated from this mixture by chromatography on cellulose (Merck microcrystalline cellulose - Avicel®) using methanol as the eluant. The purified product was obtained as a colourless solid , m.p. 158—160 °C, (Found: C, 19.02; H, 5.34; N, 5.62. $C_4H_{13}NO_7P_2$ requires C, 19.29; H, 5.26; N, 5.62%); δ_P(HCl, 5M) 9.5; δ_H(HCl, 5M) 2.99 (6H, s, NMe_2), 4.09 (2H, t, J_{PH} 10 Hz, 2-H); δ_C(HCl, 5M) 42.5 (NMe_2), 60.0 (C-2), 69.6 (t, J_{PC} 130 Hz, C-1).

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