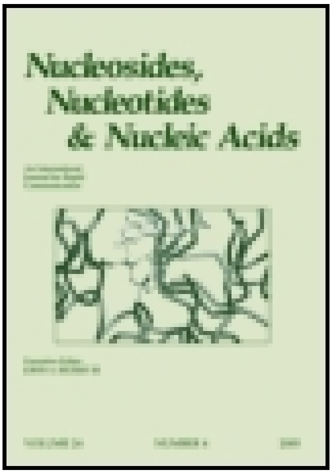
This article was downloaded by: [Temple University Libraries]
On: 04 January 2015, At: 13:34
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

A Convenient and Efficient Method for the Synthesis of Nucleoside H-Phosphonates Using a Novel Phosphonylating Agent

Zai-Wan Yang [a], Zu-Sheng Xu [a], Nan-Zhen Shen [a] & Zhi-Qiang Fang [a]

[a] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai, 200032, China

Published online: 24 Sep 2006.

To cite this article: Zai-Wan Yang, Zu-Sheng Xu, Nan-Zhen Shen & Zhi-Qiang Fang (1995) A Convenient and Efficient Method for the Synthesis of Nucleoside H-Phosphonates Using a Novel Phosphonylating Agent, Nucleosides and Nucleotides, 14:1-2, 167-173, DOI: 10.1080/15257779508014660

To link to this article: http://dx.doi.org/10.1080/15257779508014660

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

NUCLEOSIDES & NUCLEOTIDES, 14(1&2), 167-173 (1995)

A CONVENIENT AND EFFICIENT METHOD FOR THE SYNTHESIS OF NUCLEOSIDE H-PHOSPHONATES USING A NOVEL PHOSPHONYLATING AGENT

Zai-Wan Yang*, Zu-Sheng Xu, Nan-Zhen Shen
and Zhi-Qiang Fang

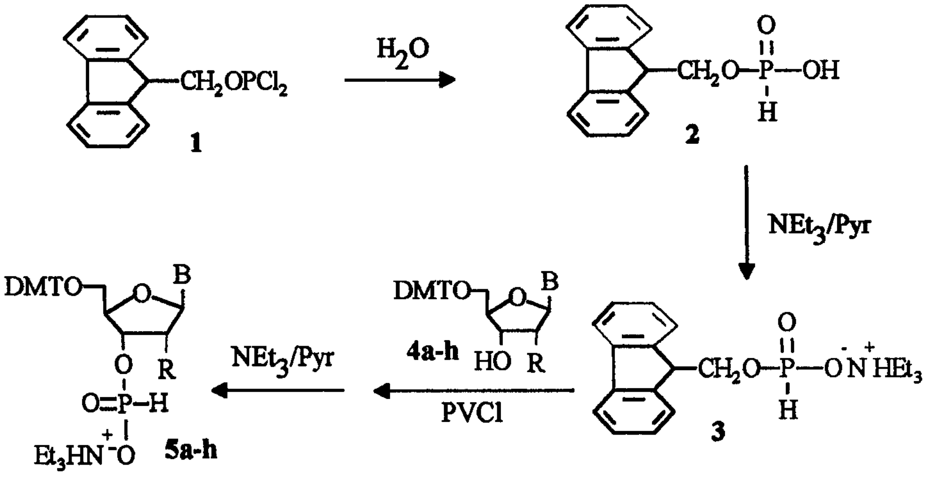
Shanghai Institute of Organic Chemistry,
Chinese Academy of Sciences, 354 Fenglin Lu, Shanghai 200032, China

Abstract: In the present paper we describe the preparation of a novel crystalline phosphonylating agent 9-fluorenemethyl phosphonic acid **2** and a convenient and efficient method for the synthesis of nucleoside H-phosphonates **5**.

The nucleoside H-phosphonate method was reported for the first time by Todd *et al.* in 1957.[1] Recently, the H-phosphonate approach has been developed into a simple, fast and efficient method for the synthesis of oligonucleotides by two groups,[2] and its advantages over the phosphoramidite approach are more and more apparently.[3]

The nucleoside 3'-H-phosphonates are key intermediates in the synthesis of oligonucleotides by the H-phosphonate approach. In the reported routes[4] for preparating H-phosphonates, unstable phosphonylating agent such as phosphorus trichloride with triazole or imidazole are usually employed. These reagents react with 5'-

protected nucleosides to form the desired 5'-protected nucleoside-3'-H-phosphonates, however, frequently (3'-3') linked undesired products are produced.



DMT = 4,4'-dimethoxytriphenylmethyl
B: a = thymine b, f = *N*-benzoyl adenine c, g = *N*-benzoyl cytosine
d, h = *N*-isobutyryl guanine e = uracil
R: a, b, c, d = H e, f, g, h = *t*-butyldimethylsilyl

We now report a convenient and efficient method for the synthesis of nucleosides H-phosphonates using a novel crystalline phosphonylating agent 9-fluorenemethyl phosphonic acid **2**.

The new phosphonylating agent is prepared by hydrolyzing compound **1** with aqueous acetonitrile solution. It readily forms needle crystals which can be stored without decrease in reactivity in a screwed vial at ambient temperature for more than one year. It has been proved that the reactivity of one-year-old compound **2** and its ^{31}P-NMR data are the same as that of freshly prepared sample. This agent reacts quickly with 5'-protected nucleosides after activation, however, since the OH group of the H-phosphonic acid is protected by the bulky fluorenemethyl group, the formation of (3'-3') linked by-products is prevented.

The nucleoside 3'-phosphonates **5a-h** were prepared as follows. First, reagent **2** was converted into its triethylammonium salt **3** with a solution of 20% triethylamine in pyridine. The protected nucleosides **4a-h** were then react with **3** in anhydrous pyridine in the presence of pivaloyl chloride, followed by removal of the 9-fluorenemethyl group from the condensation product which was completed immediately when it was coevaporated with a 20% solution of triethylamine in pyridine. Isolated yields and ^{31}P-NMR data are listed in Table 1. It can be seen that the route described above affords products in high yield.

EXPERIMENTAL

Thin layer chromatography (TLC) was carried out on silica gel HF_{254} plates (Qingdao Ocean Chemical Factory) developed with 44:5:1 CH_2Cl_2:MeOH:Et_3N and visualized using short wavelength (254 nm) UV light. Column chromatography was conducted under low pressure using silica gel H (10~40 μ, Qingdao Ocean Chemical Factory). ^{1}H- and ^{31}P-NMR spectra were recorded on BRUKER AM-300 (300 MHz) spectrometer using TMS as internal standard for ^{1}H-NMR and 85% H_3PO_4 for ^{31}P-NMR. 9-Fluorenemethanol was obtained from Shanghai Dongfeng Biochemical Factory, and 9-fluorenemethylphosphorodichloridite **1** was prepared according to reported procedure.[5] The partially protected nucleosides **4a-h** were synthesized according to published procedures[6] and coevaporated with pyridine prior to use. 2 M solution of triethylamonium bicarbonate (TEAB) was prepared by bubbling CO_2 through mixture of triethylamine and water until a homogeneous phase was formed and pH 8.2. Pyridine was distilled twice from *p*-toluenesulfonyl chloride and CaH_2 and then stored over activated 4A° molecular sieves. Acetonitrile was dried by refluxing with CaH_2 for 15 h and then distilled. Pivaloyl chloride was redistilled before use.

TABLE 1. Isolated yields and ^{31}P-NMR data (in CD_3COCD_3) of Nucleoside 3'-H-phosphonates

Compound No.	Yields (%)	^{31}P-NMR chemical shift (ppm)
5a	93	0.46
5b	94	0.31
5c	91	0.07
5d	94	0.20
5e	95	0.29
5f	93	1.00
5g	97	0.27
5h	90	2.12

9-Fluorenemethyl phosphonic acid **2**. To a stirred solution of 9-fluorenemethylphosphorodichloridite **1** (5.94 g, 20 mmol) in 120 mL acetonitrile was added dropwise 30 mL H_2O over 10 min at room temperature. After 1 h the solution was concentrated *in vacuo* to leave a white solid. The solid was then coevaporated with dry acetonitrile (3 X 50 mL) and recrystallized with the same solvent (40 mL) to give a crystalline solid **2**. Yield 4.94 g (95%). mp 118-120 °C (uncorrected). ^{31}P-NMR ($CDCl_3$) δ (ppm): 8.41, J_{PH}=708 Hz. ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.2-7.7 (8H, fluorenyl-aromatic H); 4.2-4.4 (3H, fluorenyl-H9 and -CH_2); 8.6 (1H, OH); 5.6, 8.6 (1H, $J_{H\text{-}P}$ 708.1, H-P). *Anal.* $C_{14}H_{13}O_3P$, Calcd.: C, 64.58; H, 4.94; P, 11.80. Found: C, 64.61; H, 5.04; P, 11.90.

Nucleoside 3'-H-phosphonates **5**. Compound **2** (1.1 mmol) was coevaporated with pyridine containing 20% of triethylamine (3 X 10 mL). The residue was dissolved in pyridine (5 mL), and to this solution was added **4** (1 mmol in 5 mL pyridine) and pivaloyl chloride (3 mmol). After 5 min, the reaction was quenched by addition of 1 M TEAB (0.5 mL), and the mixture was concentrated to an oil and coevaporated with pyridine containing 20% of triethylamine (3 X 10 mL). The residue was subsequently dissolved in CH_2Cl_2 (40 mL), washed with TEAB buffer (1 M, 40 mL) and dried (Na_2SO_4). The CH_2Cl_2 layer was evaporated and the residue was applied to a silica gel column and eluted with a stepwise gradient of MeOH (0-4%) in CH_2Cl_2 containing triethylamine (2%). The appropriate fractions were pooled and washed with 1 M TEAB and dried (Na_2SO_4) to give the corresponding H-phosphonates **5**. The isolated yields and the ^{31}P-NMR data are shown in Table 1, and the R_f and ^{1}H-NMR data are as follows:

data for **5a** R_f 0.57 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 7.61 (s, 1H, H6); 8.55 (s, 1/2 H, P-H), 5.38 (s, 1/2 H, P-H), and J_{PH}=617.65 Hz; 7.50-6.67 (m, 13H, DMT); 6.41 (m, 1H, H1'); 5.04 (m, 1H, H3'); 4.28 (m, 1H, H4'); 3.78 (s, 6H, 2OCH_3); 3.45 (m, 2H, 5'-CH_2); 3.08 (q, 6H, 3CH_2 of NEt_3); 2.72-2.26 (m, 2H, 2'-CH_2); 1.89 (s, 3H, 5-CH_3); 1.34 (t, 9H, 3CH_3 of NEt_3).

data for **5b** R_f 0.53 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.60 and 8.52 (2s, 2H, H8 and H2); 8.17-6.74 (m, 18H, DMT and benzoyl); 7.83 (s, 1/2 H, P-H), 5.79 (s, 1/2 H, P-H), and J_{PH}=609.04 Hz; 6.62 (m, 1H, H1'); 5.15 (m, 1H, H3'); 4.35 (m, 1H, H4'); 3.74 (s, 6H, 2OCH_3); 3.46-2.70 (m, 10H, 5'-CH_2, 3CH_2 of NEt_3 and 2'-CH_2); 1.26 (t, 9H, 3CH_3 of NEt_3).

data for **5c** R_f 0.55 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.32 (d, 1H, H6); 8.22-6.82 (m, 18H, DMT and benzoyl); 8.07 (s, 1/2 H, P-H), 5.24 (s, 1/2 H, P-H), and J_{PH}=615.14 Hz; 6.25 (m, 1H, H1'); 5.24 (d, 1H, H5); 5.06 (m, 1H, H3'); 4.32 (m, 1H, H4'); 3.82 (s, 6H, 2OCH_3); 3.53 (m, 2H, 5'-CH_2); 3.16 (q, 6H, 3CH_2 of NEt_3); 2.76-2.35 (m, 2H, 2'-CH_2); 1.32 (t, 9H, 3CH_3 of NEt_3).

data for **5d** R_f 0.56 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.02 (s, 1H, H8); 7.33 (s, 1/2 H, P-H), 5.80 (s, 1/2 H, P-H), and J_{PH}=612.31 Hz; 7.38-6.75 (m, 13H, DMT); 6.35 (t, J=6.17 Hz, 1H, H1'); 5.24 (m, 1H, H3'); 4.27 (m, 1H, H4'); 3.70 (s, 6H, $2OCH_3$); 3.52-3.24 (m, 2H, 5'-CH_2); 3.08 (q, 6H, $3CH_2$ of NEt_3); 2.98-2.62 (m, 3H, CH of iBu and 2'-CH_2); 1.22 (t, 9H, $3CH_3$ of NEt_3); 1.13 (d, 6H, $2CH_3$ of iBu).

<u>data for **5e**</u> R_f 0.59 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 7.98 (d, J=8.14 Hz, 1H, H6); 7.76 (s, 1/2 H, P-H), 5.60 (s, 1/2 H, P-H), and J_{PH}=617.65 Hz; 7.54-6.80 (m, 13H, DMT); 5.94 (d, J=3.02 Hz, 1H, H1'); 5.32 (d, 1H, H5); 4.45-4.10 (3H, H2', H3' and H4'); 3.72 (s, 6H, $2OCH_3$); 3.46 (m, 2H, 5'-CH_2); 3.12 (q, 6H, $3CH_2$ of NEt_3); 1.21 (t, 9H, $3CH_3$ of NEt_3); 0.82 (s, 9H, t-Bu); 0.18 (s, 3H, MeSi); 0.12 (s, 3H, SiMe).

<u>data for **5f**</u> R_f 0.54 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.67 and 8.58 (2s, 2H, H8 and H2); 7.82 (s, 1/2 H, P-H), 5.93 (s, 1/2 H, P-H), and J_{PH}=616.03 Hz; 8.20-6.80 (m, 18H, DMT and benzoyl); 6.23 (d, J=6.22 Hz, 1H, H1'); 5.08-4.72 (m+m, 2H, H2' and H3'); 4.38 (m, 1H, H4'); 3.76 (s, 6H, $2OCH_3$); 3.48 (m, 2H, 5'-CH_2); 3.10 (q, 6H, $3CH_2$ of NEt_3); 1.23 (t, 9H, $3CH_3$ of NEt_3); 0.85 (s, 9H, t-Bu); 0.14 (s, 3H, MeSi); 0.04 (s, 3H, SiMe).

<u>data for **5g**</u> R_f 0.57 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.46 (d, 1H, H6); 7.89 (s, 1/2 H, P-H), 5.87 (s, 1/2 H, P-H), and J_{PH}=614.56 Hz; 8.17-6.90 (m, 18H, DMT and benzoyl); 5.89 (d, J=1.45 Hz, 1H, H1'); 4.86-3.56 (6H, H2', H3', H4', 5'-CH_2 and H5); 3.70 (s, 6H, $2OCH_3$); 3.08 (q, 6H, $3CH_2$ of NEt_3); 1.24 (t, 9H, $3CH_3$ of NEt_3); 0.92 (s, 9H, t-Bu); 0.31-0.24 (2s, 6H, 2SiMe).

<u>data for **5h**</u> R_f 0.56 (CH_2Cl_2:CH_3OH:NEt_3, 44:5:1, V/V/V). ^{1}H-NMR (CD_3COCD_3) δ (ppm): 8.06 (s, 1H, H8); 7.93 (s, 1/2 H, P-H), 5.86 (s, 1/2 H, P-H), and J_{PH}=615.57 Hz; 7.42-6.75 (m, 13H, DMT); 5.96 (d, J=4.32 Hz, 1H, H1'); 5.34-4.99 (m+m, 2H, H2' and H3'); 4.33 (m, 1H, H4'); 3.71 (s, 6H, $2OCH_3$); 3.42 (m, 2H, 5'-CH_2); 3.12 (q, 6H, $3CH_2$ of NEt_3); 2.85 (m, 1H, CH of iBu);

1.26 (t, 9H, $3CH_3$ of NEt_3); 1.12 (2d, 6H, $2CH_3$ of iBu); 0.83 (s, 9H, t-Bu); 0.12 (s, 3H, MeSi); 0.02 (s, 3H, SiMe).

REFERENCES

1. Hall, R. H.; Todd, A.; Webb, R. F. *J. Chem. Soc.*, **1957**, 3291.
2. Garegg, P. J.; Regberg, T.; Stawinski, J.; Stromberg, R. *Chemica Scripta*, **1986**, 26, 59; Froehler, B. C.; Matteucci, M. C. *Tetrahedron Lett.*, **1986,** 27, 469.
3. Garegg, P. J.; Lindh, I.; Regberg, T.; Stawinski, J.; Stromberg, R. *Tetrahedron Lett.*, **1986**, 27, 4051; Tanaka, T.; Tamastukuri, S.; Kehara, M.I. *Nucleic Acids Res.*, **1987**, 15, 7235; Gao, H.; Gaffney, B.L.; Jones, R.A. *Tetrahedron Lett.*, **1991**, 32, 5477; Patil, S.V.; Mane, R.B.; Salunkhe, M.M. *Ind. J. Chem.*, **1992,** 318, 375; Battistini, C.; Fustinoni, S.; Brasca, M.G.; Borghi, D. *Tetrahedron*, **1993**, 49, 1115.
4. Sekine, M.; Hata, T. *Tetrahedron Lett.*, **1975**, 1711; Marugg, J. E.; Tromp, M.; Kuyi-Yeheskiely, E.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.*, **1986**, 27, 2661; Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.*, **1986**, 27, 2271; Sekine, M.; Narui, S.; Hata, T. *Tetrahedron Lett.*, **1988**, 29, 1037; Sakatsume, O.; Yamane, H.; Takaku, H.; Yamamoto, N. *Nucleic Acids Res.*, **1990**, 18, 3327.
5. Classen, C. A. A.; Segers, R. P. A. M.; Tesser, G. I. *Recl. Trav. Chem. Pays-Bas*, **1985**, 104, 119.
6. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.*, **1982**, 104, 1316; Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Can. J. Chem.*, **1982**, 60, 1106.

Received June 29, 1994
Accepted October 11, 1994