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Microwave-assisted cleavage of phosphate, phosphonate and phosphoramide esters

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Abstract—A mild and rapid protocol for cleavage of phosphate, phosphonate and phosphoramide esters. The scope and limitations of this microwave-assisted reaction is explored here.

Phosphorylation of proteins among other things facilitates protein–protein interactions that are important for a variety of cellular functions, including cell division.[1] Therefore, phosphate mimics viz., phosphonates and phosphoramides are valuable chemical tools to probe cellular functions.[2] For example, sphingosine-1-phosphate, is a suspect in a plethora of diseases states.[3] Due to the metabolic lability of the phosphate group on sphingosine-1-phosphate it was difficult to use sphingosine-1-phosphate as a chemical probe.[4] The recent availability of the metabolically resistant phosphonate and phosphoramide mimics of sphingosine-1-phosphates has made possible investigations into the role of sphingosine-1-phosphate in the intracellular functions leading to the disease states.[5,6] Phosphate, phosphonate and phosphoramide functionality are also used in enhancing the solubility of the drugs and in some cases they function as prodrugs.[7–10] Phosphoramides and phosphonates have also been used in the synthesis of α-aminophosphonic acids and aminoalkylphosphonic acids, respectively, as surrogates for the corresponding amino acids in biological systems.[11,12] Therefore, a mild method to introduce these functionalities into small molecule/peptides will be advantageous, for example; (1) in developing chemical tools to study phosphorylation dependent biological interactions, (2) to incorporate these as solubilizing groups in drug development and (3) particularly using the phosphoramide groups to generate prodrugs.

The phosphate, phosphonate and phosphoramide group are usually carried through multi-step synthesis as their corresponding esters. The established methodology to remove the ester protecting groups of phosphonates involves the use of trimethylsilyl bromide (TMSBr) over long reaction times.[13,14] Herein, we describe a microwave-assisted version of this methodology that reduces the reaction time to minutes and yields pure products as phosphonic acids upon work-up. In addition, we have evaluated the scope of this methodology by extending it to phosphate and phosphoramide esters.

Microwave-assisted organic transformations reduce chemical reaction times from hours to minutes, improve reproducibility and result in high yielding reactions.[15,16] During the cleavage of diethyl-(3-phenyl-allyl)-phosphonate ester by TMSBr, we found that the reaction times were reduced from 8 h at room temperature to 10 min at 100 °C in the microwave (CEM-Discover mono-mode microwave apparatus). This observation led us to investigate the use of microwave for cleavage of phosphonate ethyl esters.

The readily available diethyl-(3-phenyl-allyl)-phosphonate ester was chosen as the substrate to optimize the reaction conditions. Dielectric loss (ε'') reflects the efficiency of a solvent to convert microwave energy to thermal energy.[17] Three solvents with varying ε'' were chosen for the optimization. Table 1 summarizes the optimization of solvent and temperatures for the cleavage reaction. Time course and ratio of reagents required for completion of reaction in the microwave was monitored by TLC.

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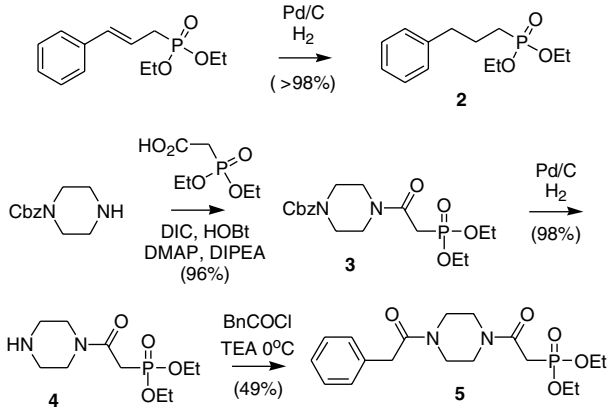
Table 1. Optimization of reaction conditions for microwave-assisted cleavage of phosphonate esters

1

Entry	Solvent	Dielectric loss (ε'')	Microwave (W read back)	Temperature (°C)	Time (min)	Yield[a] (%)
1	$PhCH_3$	0.096	175	100	10	65
2	THF	0.348	250	100	10	55
3	CH_3CN	2.325	250	100	10	>95
4	CH_3CN	2.325	220	80	10	>95
5	CH_3CN	2.325	100	60	10	>95
6	CH_3CN	2.325	35	40	10	>95
7	CH_3CN	2.325	No	rt	480	92
8	CH_3CN	2.325	No	40	400	93

[a] Yield of isolated product.

TMSBr (2 equiv) and a reaction time of 10 min were required to drive the reaction to completion in the microwave. The reactions were quenched with a 95:5 methanol–water mixture and concentrated to yield the final product. The microwave was set to deliver 250 W of power; however, the read back on the instrument (see Supporting data) varied with solvents and temperatures (Table columns 2, 4 and 5). Since the reaction proceeds well at 40 °C and 35 W of power, all further experiments were carried out at 40 °C with a power setting of 50 W. Based on the isolated yields, it was clear that acetonitrile was the solvent of choice and the reaction reaches completion at 40 °C. By comparing entries 6–8 (Table 1), it is clear the reaction proceeds faster under the microwave conditions without compromising product yields.



Scheme 1. Synthesis of phosphonate esters **2** and **5**.

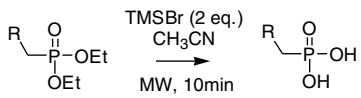
To explore the scope of this reaction, a combination of commercially available and easily accessible starting phosphonates was used as their corresponding ethyl esters. Compound **2** was synthesized by catalytic Pd/C hydrogenation of the corresponding olefin **1**.[18] The reaction proceeds smoothly and the product was isolated in a quantitative yield. The starting material **5** was synthesized in three steps starting from readily available Cbz-protected piperizine. Cbz-piperizine was coupled to (diethoxy-phosphoryl)-acetic acid using standard peptide coupling procedures to yield **3**.[19] The Cbz group on the resulting compound **3** was deprotected by hydrogenation to yield **4**.[20] In the final step, the free amine on the piperizine was reacted with phenylacetyl chloride to yield the desired phosphonate ester **5**.[21] Scheme 1 illustrates the synthesis of **2** and **5**. Compounds in Table 2, entries 3 and 4 are commercially available and were used without further purification. Table 2 summarizes the yields of the cleavage reaction of the phosphonates. The microwave-assisted reaction is mild as it tolerates labile functional groups and is a high yielding reaction.

We then turned our attention to phosphate and phosphoramide esters. The phosphate and phosphoramide esters were prepared in good yields from the corresponding alcohol or amine and diethylchlorophosphate.[12] The reaction proceeds smoothly at 0 °C in 3–6 h depending on the substrates. The reactions were monitored for completion by TLC. Table 3 summarizes the yields of the ester and the product obtained in these reactions.

Phosphoramides generated from the primary amines undergo cleavage to give the corresponding phosphoramidic acids in high yields (Table 3, entries 1, 2, 4 and 5). In a control experiment, benzyl phosphoramide (Table 3, entry 4) when treated with TMSBr takes 6 h at 40 °C in the hood reach completion and the corresponding acid was isolated in >90% yield. Although the yields in the hood and the microwave are comparable, it is clear that the reaction in the microwave is faster.

We then explore the scope and limitations of this microwave-assisted cleavage of esters. Phosphoramide esters generated from alkyl, propargyl, benzyl and aryl amines were cleaved rapidly to their corresponding phosphoramide in high yields (Table 3; entries 1, 2, 4 and 7). A substrate containing a benzyl ester and a primary phosphoramide ester (Table 3, entry 3), when subjected to microwave-assisted cleavage by TMSBr, yields a mixture of products. The desired product was isolated in modest yield, however, the mixture includes products in which the benzyl ester was also cleaved. This suggests that the compounds containing benzyl esters might not be stable to the reaction conditions. Also the phosphor-

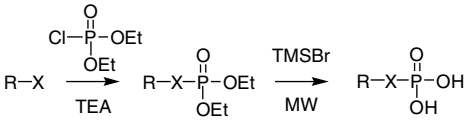
Table 2. Microwave-assisted cleavage of phosphonate esters

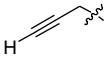
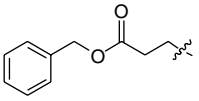
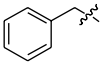
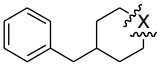
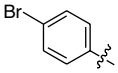
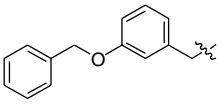
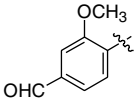


Entry	R	Yield[a] (%)
1	**2**	96
2	**5**	94
3	$-CH_2CN$	92
4	$-CH_2CH_2Br$	96

[a] Yield of isolated product.

Table 3. Synthesis of phosphate and phosphoramide esters followed by microwave-assisted cleavage[22]



Entry	R	X	Ester yield[a] (%)	Product yield[a] (%)
1		N	47	94
2		N	63	89
3		N	51	27[b]
4		N	68	93
6		N	73	76[c]
7		N	21	96
8		O	42[d]	70[e]
9		O	57	97

[a] Yield of isolated product.
[b] Isolated product from a mixture.
[c] Isolated yield of 4-benzyl piperidine.
[d] Reaction required catalytic amount of DMAP.
[e] Isolated yield of benzyloxybenzylbromide.

amide ethyl ester generated from 4-benzylpiperidine, was not stable to TMSBr (Table 3, entry 6). The product isolated from this reaction was 4-benzylpiperidine suggesting that the P–N bond is also susceptible to TMSBr cleavage (Table 3, entry 6). In the case of phosphate esters, cleavage of the benzyl phosphate ester yields the corresponding benzyl bromide, probably thorough an S_N2 type displacement of the phosphate group by the bromide (Table 3, entry 8). This assumption is supported by the smooth conversion of an aryl phosphate ester to the corresponding aryl phosphate (Table 3, entry 9).

In conclusion, we have identified a rapid microwave-assisted method to generate phosphates, phosphonates and phosphoramides from their corresponding ethyl esters. The cleavage of the esters proceeds faster under microwave conditions. Although this methodology works well for phosphonate esters, phosphoramide esters from primary amines and aryl phosphate esters, it is not suitable for benzyl phosphate ester and phosphoramide esters generated from secondary amine. To the best of our knowledge, this is the first report that describes the cleavage of phosphoramide esters using TMSBr.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.06.126.

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22. *General procedure for synthesis of phosphate and phosphoramide esters*: Diethyl chlorophosphate (1 equiv) was added drop wise to a cooled (0 °C) solution of phenol or amine (1 equiv) and triethylamine (1.2 equiv) in dry CH_2Cl_2 under nitrogen. The reaction was warm to room temperature and stirring was continued at until completion of reaction (3–6 h). The reaction mixture was extracted with 1 M HCl and a saturated aqueous solution of $NaHCO_3$. The organic phase was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by column chromatography. Table 3, entry **1** ester. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (t, $J = 7.2$ Hz, $2 \times CH_3$, 6H), 1.59–1.63 (m, 6H), 1.80–1.81 (m, 6H), 2.05–2.10 (m, 3H), 4.07 (q, $J = 7.2$ Hz, $2 \times CH_2$, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 62.38, 44.72, 36.43, 30.06, 16.68. ^{15}P NMR (121 MHz, $CDCl_3$) δ: 8.01. MS (ESI) $C_{14}H_{26}NO_3P$ $(M+H)^+$ 288.56. Table 3, entry **9** ester. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.39 (t, $J = 7.2$ Hz, $2 \times CH_3$, 6H), 3.95 (s, 3H), 4.28 (q, $J = 7.2$ Hz, $2 \times CH_2$, 4H), 7.47–7.48 (m, 3H), 9.92 (br s, CHO, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 190.99, 150.4, 145.3, 132.1, 125.31, 121.60, 111.03, 65.24, 56.42. ^{15}P NMR (121 MHz, $CDCl_3$) δ: −5.56. MS (ESI) $C_{12}H_{17}O_6P$ $(M+H)^+$ 289.63.
Table 1, entry **5** ester. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (t, $J = 6.9$ Hz, $2 \times CH_3$, 6H), 2.99–3.06 (m, 2H), 3.29–3.68 (m, 8H), 3.69–3.72 (m, 2H), 4.09 (q, $J = 6.9$ Hz, $2 \times CH_2$, 4H), 7.18–7.28 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 169.77, 163.61, 134.71, 129.05, 128.60, 127.18, 63.08, 46.94, 42.10, 41.74, 34.50, 32.94, 16.72. MS (ESI) $C_{18}H_{27}N_2O_5P$ $(M+H)^+$ 383.48.
General procedure for microwave-assisted cleavage of phosphate, phosphonate and phosphoramide esters: A septum-sealed microwave tube charged with phosphate or phosphonate or phosphoramide esters (1 equiv) and trimethylsilylbromide (2 equiv) in CH_3CN was irradiated in a CEM-Discover mono-mode microwave cavity (50 W, 40 °C, 10 min). After completion of the reaction indicated by TLC, the reaction mixture was quenched with CH_3OH–H_2O (95:5) and concentrated to give the corresponding phosphates or phosphonates or phosphoramidic acids in good yields. Table 2, entry **2**. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.90–2.98 (m, 2H), 3.20–3.40 (m, 8H), 3.70–3.72 (m, 2H), 7.18–7.26 (m, 5H), 8.73 (br s, $2 \times OH$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 169.66, 165.02, 136.33, 129.57, 128.94, 126.99, 49.34, 47.10, 42.18, 36.87. ^{15}P NMR (121 MHz, DMSO-d_6) δ: 17.35. MS (ESI) $C_{14}H_{19}N_2O_5P$ $(M+H)^+$ 327.28. Table 3, entry **1**. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.54–1.75 (m, 6H), 1.73–1.75 (m, 6H), 2.48–2.49 (m, 3H), 7.74 (br s $2 \times OH$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 29.16, 35.91, 40.90. ^{15}P NMR (121 MHz, DMSO-d_6) δ: 0.90. MS (ESI) $C_{10}H_{18}NO_3P$ $(M+H)^+$ 232.06. Table 3, entry **2**. ^{1}H NMR (300 MHz, CD_3OD) δ: 3.09 (s, 1H), 3.21 (br s, 1H, NH), 3.62 (m, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ: 76.52, 75.03, 29.02. ^{15}P NMR (121 MHz, CD_3OD) δ: 0.904. MS (ESI) $C_3H_6NO_3P$ $(M+H)^+$ 136.62. Table 3, entry **4**. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 4.03 (s, 2H), 7.36–7.45 (m, 5H), 8.17 (br s, $2 \times OH$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 138.12, 129.50, 129.29, 128.01, 40.35. ^{15}P NMR (121 MHz, DMSO-d_6) δ: 1.086. MS (ESI) $C_7H_{10}NO_3P$ $(M+H)^+$ 188.28. Table 3, entry **7**. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.26 (d, $J = 8.4$ Hz, 2H), 7.64 (d, $J = 8.7$ Hz, 2H), 9.88 (br s, $2 \times OH$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 133.38, 133.21, 125.24, 120.22. ^{15}P NMR (121 MHz, DMSO-d_6) δ: 0.88. MS (ESI) $C_6H_7BrNO_3P$ $(M+H)^+$ 253.52. Table 3, entry **9**. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 3.82 (s, 3H), 7.41–7.48 (m, 3H), 9.86 (s, 1H), 10.2 (br s, $2 \times OH$). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 192.29, 151.42, 146.39, 133.26, 124.51, 121.07, 112.37, 56.55. ^{15}P NMR (121 MHz, DMSO-d_6) δ: −5.49. MS (ESI) $C_8H_9O_6P$ $(M+H)^+$ 234.01.