Efficient and divergent synthesis of cyclophosphamide analogues from 2-arylamino-3-acetyl-5,6-dihydro-4*H*-pyrans†

Dexuan Xiang,[a] Peng Huang,[b] Kewei Wang,[a] Guangyuan Zhou,[b] Yongjiu Liang[b] and Dewen Dong*[ab]

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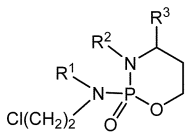
A facile and efficient one-pot synthesis of substituted cyclophosphamidic chlorides and their analogues has been developed from readily available enaminones, 2-arylamino-3-acetyl-5,6-dihydro-4*H*-pyrans.

Cyclophosphamide **1a** was introduced in tumor therapy in 1958 and is until now the most important clinically used antitumor and immunosupressive agent (Chart 1).[1,2] It is becoming clear that monooxidation of cyclophosphamide **1a** to 4-hydroxycyclophosphamide **1b** is essentially responsible for the activation of this agent by the drug-metabolizing enzymes of the liver.[3] Cyclophosphamide analogues of particular clinical interest include ifosfamide **1c** and trofosfamide **1d**.[4] So far, numerous structural modifications have been carried out in attempts to improve the drug's therapeutic index and elucidate their mechanism of action. The notable approach for the synthesis of cyclophosphamides was established by Takamizawa and coworkers *via* the ozonolysis of *O*-3-butenylphosphorodiamidates.[5] Other methods were reported by the reaction of phosphoramidic dichlorides with 1,3-amino alcohols or 3-hydroxyamide.[6]

Recently, we reported the efficient synthesis of substituted pyridin-2(1*H*)-ones *via* the Vilsmeier–Haack reactions of a variety of β-oxo amide derivatives, 1-acyl and 1-carbamyl cyclopropanes, 5,6-dihydro-4*H*-pyrans, α-monosubstituted β-oxo amides and α-unsubstituted β-oxo amides.[7] Furthermore, we achieved one-pot synthesis of fully substituted 1*H*-pyrazoles from the oximes of 1-acyl and 1-carbamyl cyclopropanes under Vilsmeier conditions ($POCl_3$/DMF).[8] When DMF was replaced with CH_2Cl_2, the same substrates, cyclopropyl oximes, afforded fully substituted isoxazoles in high yields. The results suggested that $POCl_3$, being a reagent,[9] showed different reaction behavior from the Vilsmeier reagent, $POCl_3$/DMF. Inspired by these results and in continuation with our research interests regarding the development of new approaches for the synthesis of highly valuable heterocycles, we were interested in examining the reaction behavior of the readily available enaminones toward $POCl_3$ in CH_2Cl_2. As a result of these studies, we provide a facile and efficient one-pot synthesis of unsaturated cyclophosphamidic chlorides, a new ring system, and their further synthetic transformations into cyclophosphamidic amides and esters. In the present work, we wish to report our results and the possible mechanism involved.



1

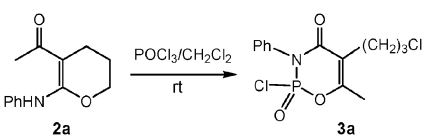
1	R^1	R^2	R^3
1a	$(CH_2)_2Cl$	H	H
1b	$(CH_2)_2Cl$	H	OH
1c	H	$(CH_2)_2Cl$	H
1d	$(CH_2)_2Cl$	$(CH_2)_2Cl$	H

Chart 1

The substrates, 5,6-dihydro-4*H*-pyrans **2**, were synthesized from commercially available β-oxo amides and 1,3-dibromoethane in the presence of K_2CO_3 in DMF in excellent yields (up to 96%) according to our published procedure.[7b] With substrates **2** in hand, we selected 2-phenylamino-3-acetyl-5,6-dihydro-4*H*-pyran **2a** as the model compound to examine its behavior. Thus, the reaction of **2a** with $POCl_3$ (3.0 equiv.) was first attempted in CH_2Cl_2 at room temperature. As monitored by TLC, the reaction proceeded smoothly and furnished a white solid after workup and subsequent purification by column chromatography of the resulting mixture. The product was characterized as a cyclophosphamidic chloride (90% yield) on the basis of its spectral and analytical data (Scheme 1). The structure of **3a** was further confirmed by the X-ray single-crystal analysis (Fig. 1).‡

The optimization of the reaction conditions, including reaction temperature and the ratio of $POCl_3$ to **2a** was then investigated. A series of experiments revealed that 1.2 equiv. of $POCl_3$ was effective for the synthesis of **3a**, and the yield of **3a** reached 91% when the reaction of **2a** with 1.2 equiv. of $POCl_3$ was performed in CH_2Cl_2 at room temperature for 40 min (Table 1, entry 1). Under the optimal conditions, we carried out a series of reactions of **2** aiming to determine its scope with respect to the amide moiety. As shown in Table 1, the ring-opening/recyclization proved to be suitable for **2b–e** bearing varied *N*-aryl groups, affording the corresponding substituted cyclophosphamidic chlorides **3b–e** in very high yields (Table 1, entries 2–4). It is worth mentioning that the cyclophosphamidic chlorides **3** are stable when subjected to the aqueous workup process (duration: 1.0 h).

Therefore, we provided a simple and efficient one-pot synthesis of substituted cyclophosphamidic chlorides. It was assumed that the cyclophosphamidic chlorides **3** were formed *via* tandem ring-opening mediated by $POCl_3$ and an intramolecular cyclization as depicted in Scheme 2.[6d,7b]



2a **3a**

Scheme 1 Reaction of **2a** with $POCl_3$ in CH_2Cl_2.

[a] *Department of Chemistry, Northeast Normal University, 130024 Changchun, China*
[b] *Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, 130022 Changchun, China.*
E-mail: dongdw663@nenu.edu.cn; Fax: +86 431 8509 8635

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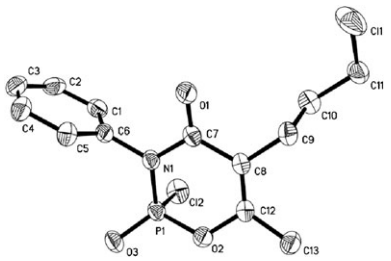
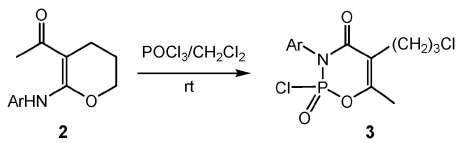


Fig. 1 ORTEP drawing of **3a**.

Table 1 Synthesis of cyclophosphamidic chlorides **3**[a]

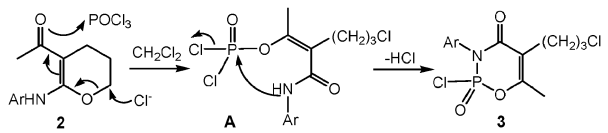


Entry	**2**	Ar	*t*/min	**3**	Yield[b] (%)
1	**2a**	Ph	40	**3a**	91
2	**2b**	4-MeC_6H_4	40	**3b**	89
3	**2c**	4-$MeOC_6H_4$	30	**3c**	92
4	**2d**	2-$MeOC_6H_4$	60	**3d**	87
5	**2e**	4-ClC_6H_4	45	**3e**	90

[a] *Reagents and conditions*: **2** (1.0 mmol), $POCl_3$ (1.2 mmol), CH_2Cl_2, rt, 0.5–1.0 h. [b] Isolated yield.

It is worth noting that the cyclophosphamidic chlorides **3** possess very rich functionality, such as α,β-unsaturated carbonyl and chloropropyl groups, and in particular a phosphorus chloride moiety, which may render them extremely versatile as valuable synthetic scaffolds in the preparation of natural and synthetic compounds with important biological and pharmacological activities. For example, the amidation product of **3** upon hydrogenation may lead to the same core structure of cyclophosphamide **1**.

Inspired by these, we next performed the reactions of cyclophosphamidic chlorides **3** with selected amines with the aim to synthesize cyclophosphamidic amides. Thus, the reaction of **3a** and ethyl amine (aq, 1.1 equiv.) was conducted in the presence of triethyl amine (1.2 equiv.) in CH_2Cl_2 at room temperature for 1.0 h. Workup and purification by column chromatography of the resulting mixture furnished a product, which was characterized as a cyclophosphamidic amide **4a-1** (92% yield) on the basis of its spectral and analytical data (Table 2, entry 1). Under the identical conditions, a range of cyclophosphamidic chlorides **3b–e** containing different *N*-aryl groups were reacted with ethyl amine, and the corresponding cyclophosphamidic amides **4** were obtained in very high yields (Table 2, entries 2–5). The versatility of the synthesis of cyclophosphamidic amides **4** was further evaluated by reacting **3a** with selected aliphatic and aromatic amines, such as methylamine (aq), aniline and benzylamine (Table 2, entries 6–8).



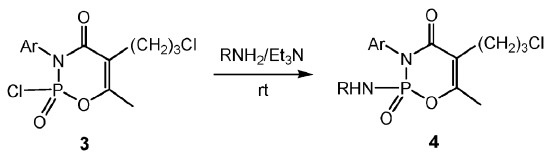
Scheme 2 Plausible mechanism for the synthesis of cyclophosphamidic chlorides **3**.

Next, we examined the reactions of **3a** and alcohols with respect to the synthesis of cyclophosphamidic esters. When **3a**, methanol (1.1 equiv.) and pyridine (1.2 equiv.) were subjected to CH_2Cl_2 at room temperature for 1.0 h (monitored by TLC), the reaction proceeded smoothly and furnished a white solid after workup and purification by column chromatography of the resulting reaction mixture. The product was characterized as a cyclophosphamidic ester **5a-1** (90% yield) on the basis of its spectral and analytical data (Table 3). In the same fashion, cyclophosphamidic esters **5a-2**, **5a-3** and **5a-4** were obtained in excellent yields by reacting **3a** with ethanol, phenol and benzyl alcohol, respectively (Table 3).

In fact, a one-pot synthesis of substituted cyclophosphamidic amides **4** from enaminones **2** was attempted. In a representative experiment, **2a** was treated with $POCl_3$ (1.2 equiv.) in CH_2Cl_2 at room temperature for 1.0 h, then triethyl amine (4.0 equiv.) and ethyl amine (1.5 equiv.) were loaded in tandem sequence under stirring. The reaction mixture was stirred for further 1.0 h to afford **4a-1** in high yield (Scheme 3). Similarly, a one-pot synthesis of substituted cyclophosphamidic ester **5a-1** from enaminone **2a** was achieved by treatment of **2a** with $POCl_3$ (1.2 equiv.) in CH_2Cl_2, followed by addition of pyridine (4.0 equiv.) and methanol (1.2 equiv.).

In summary, a facile and efficient one-pot synthesis of substituted cyclophosphamidic chlorides **3** has been developed from readily available enaminones **2** in the presence of $POCl_3$ in

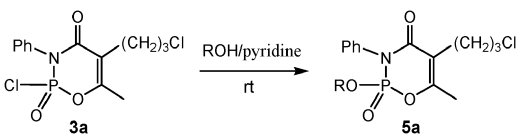
Table 2 Synthesis of cyclophosphamidic amides **4**[a]



Entry	**3**	Ar	R	**4**	Yield[b] (%)
1	**3a**	Ph	Et	**4a-1**	92
2	**3b**	4-MeC_6H_4	Et	**4b-1**	91
3	**3c**	4-$MeOC_6H_4$	Et	**4c-1**	89
4	**3d**	2-$MeOC_6H_4$	Et	**4d-1**	90
5	**3e**	4-ClC_6H_4	Et	**4e-1**	93
6	**3a**	Ph	Me	**4a-2**	92
7	**3a**	Ph	Ph	**4a-3**	87
8	**3a**	Ph	Bn	**4a-4**	88

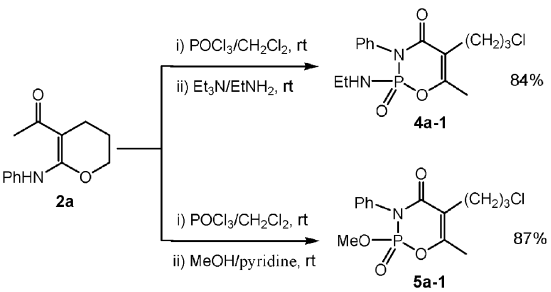
[a] *Reagents and conditions*: **3** (1.0 mmol), RNH_2 (1.1 mmol), Et_3N (1.2 mmol), CH_2Cl_2, rt, 1.0–1.5 h. [b] Isolated yields.

Table 3 Synthesis of cyclophosphamidic esters **5a**[a]



Entry	R	*t*/h	**5a**	Yield (%)[b]
1	Me	1.0	**5a-1**	90
2	Et	1.5	**5a-2**	93
3	Ph	1.5	**5a-3**	94
4	Bn	1.0	**5a-4**	92

[a] *Reagents and conditions*: **3a** (1.0 mmol), ROH (1.1 mmol), pyridine (1.2 mmol), CH_2Cl_2, rt, 1.0–1.5 h. [b] Isolated yields.



Scheme 3 One-pot synthesis of cyclophosphamidic amide (**4a-1**) and ester (**5a-1**).

CH_2Cl_2. As synthetic scaffolds, some of the newly synthesized cyclophosphamidic chlorides were employed in the reactions with selected amines and alcohols affording the corresponding cyclophosphamidic amides **4** and esters **5**, respectively, in high yields. Moreover, one-pot synthesis of cyclophosphamidic amides **4** and esters **5** from enaminones **2** has been achieved in high yields. Associated with readily available starting materials, mild conditions, high yields, and potential utility of the products, the one-pot synthetic protocol for cyclophosphamide analogues is very attractive, in particular for library synthesis. The potential utilization and extension of the scope of the methodology and the evaluation of biological activity of the novel products are currently under investigation in our laboratory.

Notes and references

‡ *Typical procedure for the synthesis of cyclophosphamidic chlorides* **3** (**3a** *as an example*): To a solution of **2a** (217 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at room temperature was added $POCl_3$ (1.2 mmol) in one portion. The mixture was stirred at room temperature for 40 minutes. After substrate **2a** was consumed as indicated by TLC, the mixture was then poured into saturated aqueous NaCl (20 mL), which was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with water (3× 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, petroleum ether–diethyl ether = 2 : 1) to give **3a** as a white solid (303 mg, 91%).
Selected data for **3a**: White solid: mp 94–95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.94–2.02 (m, 2H), 2.33 (s, 3H), 2.52–2.65 (m, 2H), 3.54–3.61 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.46–7.51 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.8, 24.5, 31.5, 44.6, 114.4, 129.3, 129.6, 129.9, 131.9, 157.1, 163.1; IR (KBr) 1691.8, 1653.0, 1490.1, 1308.1, 1238.8, 1123.9, 1072.6, 945.0, 608.6, 543.5 cm^{-1}; Anal. Calc. for $C_{13}H_{14}Cl_2NO_3P$: C, 46.73; H, 4.22; N, 4.19. Found: C, 46.60; H, 4.28; N, 4.14. MS m/z Calc.: 334.1; Found: 334.1 [M$^+$].
Crystal data for **3a**: $C_{13}H_{14}Cl_2NO_3P$, white crystal, M = 334.12, monoclinic, $P2_1/n$, a = 9.560(3), b = 10.346(3), c = 15.722(5) Å, β = 104.543(5)°, V = 1505.2 (8) Å^3, Z = 4, T = 293(2), $F000$ = 688, $R1$ = 0.0520, $wR2$ = 0.1501.
Typical procedure for the preparation of **4** *from cyclophosphamidic chlorides* **3** (**4a-1** *as example*): To a solution of **3a** (333 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at room temperature was added ethylamine (aq, 1.1 mmol) and triethylamine (1.2 mmol) in one portion. The mixture was stirred at room temperature for 1.0 h. After substrate **3a** was consumed as indicated by TLC, the mixture was then poured into saturated aqueous NaCl (20 mL), which was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with water (3 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, petroleum ether–diethyl ether = 2 : 1) to give **4a-1** as a white solid (315 mg, 92%).
Selected data for **4a-1**: White solid: mp 125–126 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.97 (t, J = 7.0 Hz, 3H), 1.93–1.98 (m, 2H), 2.26 (s, 3H), 2.44–2.57 (m, 2H), 2.81–2.85 (m, 1H), 2.86–2.96 (m, 2H), 3.56 (t, J = 6.0 Hz 2H), 7.35 (d, J = 7.0 Hz, 2H), 7.39 (d, J = 7.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.7, 19.0, 24.4, 31.7, 36.7, 44.2, 111.9, 128.7, 128.9, 129.5, 134.5, 157.3, 165.0; IR (KBr) 3221.8, 1675.3, 1455.6, 1392.9, 1257.3, 1121.9, 1073.0, 985.7, 695.6, 555.0 cm^{-1}; Anal. Calc. for $C_{15}H_{20}ClN_2O_3P$: C, 52.56; H, 5.88; N, 8.17. Found: C, 52.42; H, 5.97; N, 8.23.
Typical procedure for the preparation of **5** *from cyclophosphamidic chlorides* **3** (**5a-1** *as example*): To a solution of **3a** (333 mg, 1.0 mmol) in CH_2Cl_2 (5 mL) at room temperature was added methanol (1.1 mmol) and pyridine (1.2 mmol) in one portion. The mixture was stirred at room temperature for 1.0 h. After the substrate **3a** was consumed as indicated by TLC, the mixture was then poured into saturated aqueous NaCl (20 mL), which was extracted with dichloromethane (3 × 20 mL). The combined organic phases were washed with water (3 × 20 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography (silica gel, petroleum ether–diethyl ether = 2 : 1) to give **5a-1** as a white solid (296 mg, 90%).
Selected data for **5a-1**: White solid: mp 100–101 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.94–1.98 (m, 2H), 2.27 (s, 3H), 2.51 (t, J = 7.5 Hz, 2H), 3.55–3.58 (m, 2H), 3.79 (d, J = 12 Hz, 3H), 7.32 (d, J = 7.5 Hz, 2H), 7.42 (d, J = 7.0 Hz, 1H), 7.46 (d, J = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 18.9, 24.5, 31.6, 44.8, 56.0, 112.3, 129.2, 129.3, 129.4, 129.8, 133.2, 157.0, 164.1; Anal. Calc. for $C_{14}H_{17}ClNO_4P$: C, 51.00; H, 5.20; N, 4.25. Found: C, 51.13; H, 5.28; N, 4.17.

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