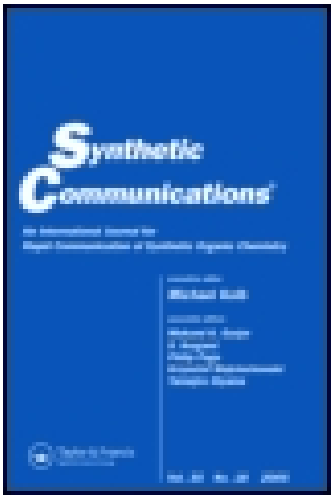


Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lsyc20

Synthesis of Novel Coumarin-7,8-cyclophosphoramide Analogs

Xiaolan Chen [a], Yadong Zhou [a], Lingbo Qu [a b], Xiangqian Liu [a], Jinwei Yuan [b] & Yufen Zhao [a c]

[a] Department of Chemistry, Zhengzhou University, Zhengzhou, China

[b] Henan University of Technology, Zhengzhou, China

[c] Key Laboratory for Bioorganic Phosphorus Chemistry, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing, China

Published online: 08 Jun 2010.

To cite this article: Xiaolan Chen, Yadong Zhou, Lingbo Qu, Xiangqian Liu, Jinwei Yuan & Yufen Zhao (2010) Synthesis of Novel Coumarin-7,8-cyclophosphoramide Analogs, Synthetic Communications: An International Journal for Rapid Communication of Synthetic Organic Chemistry, 40:13, 1992-1997, DOI: 10.1080/00397910903219369

To link to this article: http://dx.doi.org/10.1080/00397910903219369

Synthetic Communications®, 40: 1992–1997, 2010

ISSN: 0039-7911 print/1532-2432 online
DOI: 10.1080/00397910903219369

Taylor & Francis
Taylor & Francis Group

SYNTHESIS OF NOVEL COUMARIN-7,8-CYCLOPHOSPHORAMIDE ANALOGS

Xiaolan Chen,[1] Yadong Zhou,[1] Lingbo Qu,[1,2] Xiangqian Liu,[1] Jinwei Yuan,[2] and Yufen Zhao[1,3]

[1]*Department of Chemistry, Zhengzhou University, Zhengzhou, China*
[2]*Henan University of Technology, Zhengzhou, China*
[3]*Key Laboratory for Bioorganic Phosphorus Chemistry, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing, China*

Coumarin-7,8-cyclophosphoramide derivatives were conveniently prepared by a facile method. 7-Hydroxycoumarin was aminomethylated regioselectively at the 8-C position using the Mannich reaction. The newly formed 8-(N-alkylaminomethyl) coumarins were then coupled with bis-β-chloroethyl dichlorophosphamide to form coumarin-7,8-cyclophosphoramide analogs. An efficient, highly regioselective method to synthesize coumarin-7,8-cyclophosphoramide derivatives is provided, and the approach has the merits of mild reaction conditions.

Keywords: Coumarin-7,8-cyclophosphoramide; cyclophosphoramide; 7-hydroxycoumarin; Mannich reaction

INTRODUCTION

Coumarin (1,2-benzopyrone) is a widely distributed natural product with low human toxicity.[1] Coumarin and its derivatives exhibit different biological and pharmacological activities including anticoagulant, estrogenic, dermal photosensitizing, vasodilator, molluscacidal, anthelmintic, sedative, hypnotic, analgesic, hypothermic,[2] antimicrobial,[3] anti-inflammatory,[4] antifungal,[5] and antiulcer[6] activities. In vitro, coumarin and its derivatives inhibit the proliferation of several human tumor cell lines; namely, coumarin retards the development of renal[7] and prostate carcinoma[8,9] and prevents the recurrence of melanoma.[10] Coumarin may be a prodrug, and its major biotransformed product is 7-hydroxycoumarin (Fig. 1a).[11] 7-Hydroxycoumarin also exhibits cytotoxic effects against the lung adenocarcinoma cell lines KB,[9] A549,[12] SK-LU-1,1.3.15, 3A5A, and A-427.[13]

Cyclophosphamide (Fig. 1b), one of the most successful anticancer agents developed over the past few decades,[14] was used to treat various types of cancer

Received September 11, 2008.
Address correspondence to Xiaolan Chen, Department of Chemistry, Zhengzhou University, Zhengzhou 450052, China. E-mail: chenxl@zzu.edu.cn

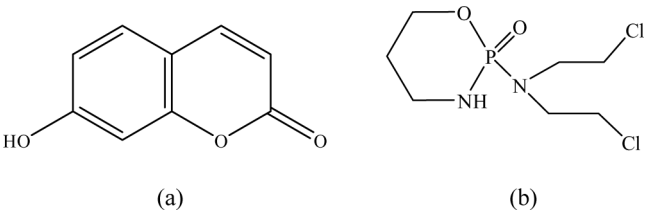
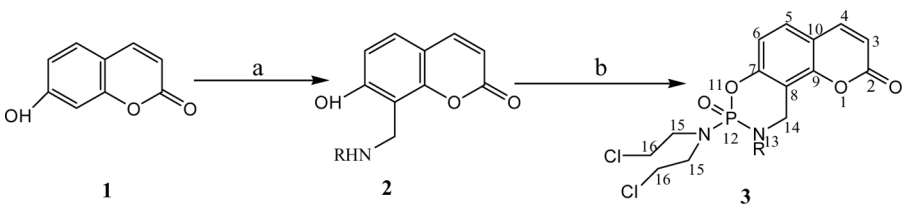


Figure 1. (a) Structure of 7-hydroxycoumarin and (b) structure of cyclophosphamide.



Scheme 1. Synthesis of title coumarin–cyclophosphamide analogs. Reagents and reaction conditions: (a) HCHO (1 equiv.), RNH_2 (1 equiv.), reflux 8–12 h; (b) bis-β-chloroethyl dichlorophosphamide (1 equiv.), Et_3N (2 equiv.), reflux 3–4 h.

and some autoimmune disorders as a prodrug. Given the biological importance of coumarin and cyclophosphamide, two pharmic groups were coupled to form new cyclophosphoramide analogs of coumarin, which would be favorable in achieving some specificity of pharmacological action in view of the development of effective clinical anticancer drugs.

The Mannich reaction is an important organic reaction, with important applications in the design and synthesis of medicine. The Mannich reaction is one method for chemical modification that introduces a basic function into a molecule. It develops a new field for the modification of natural products, which attracts increasing numbers of researchers. To search for new compounds with higher antitumor activities and lower toxicity, in this article a convenient procedure is introduced to synthesize new cyclophosphamide analogs of coumarin via the Mannich reaction and cyclized reaction (Scheme 1). The structures of these compounds were confirmed by electrospray ionization–mass spectrometry (ESI-MS), high-resolution mass spectrometry (HR-MS), NMR (including two-dimensional NMR), and infrared (IR).

RESULTS AND DISCUSSION

In the present study, five Mannich bases were successfully synthesized starting from 7-hydroxycoumarin **(1)** with formaldehyde and suitable amine in tetrahydrofuran (THF). It is generally known that the reaction pathway of the Mannich reaction depends on the nucleophilicity of substrate. The Mannich reaction, under present experimental conditions, was found to proceed regioselectively, favoring attack at 8-C of 7-hydroxycoumarin. Considering the Mannich reaction begins from electrophilic attack, we calculated the comparison of density of charges of 8-C and 6-C. It is well known that the region with heavy density of charges is prone to electrophilic

Table 1. Series of coumarin-7,8-analogs (**3**)

Entry	R	Yield (%)
3a	$CH_2CH_2CH_3$	89
3b	$CH(CH_3)_2$	88
3c	$CH_2CH_2CH_2CH_3$	90
3d	$C(CH_3)_3$	87
3e	$CH(CH_2)_4CH_2$	85

reaction. The calculated result shows that the net charge of 8-C is more than that of 6-C. Therefore, 8-C is supposed to be the main reaction site. This conclusion was also confirmed by two-dimensional NMR of compound **3d**. For example, the heteronuclear multiple bond correlation (HMBC) spectrum of compound **3d** showed that 7-C at δ 153.8, 8-C at δ 115.3, and 9-C at δ 149.9 are related, through three bonds, to 14-H at δ 4.36–4.29 and 4.68–4.60. If the Mannich reaction occurred at 6-C, there would be any correlation among 8-C, 9-C, and 14-H. HMBC spectrum also showed that 7-C at δ 153.8 is related, through two bonds, to 5-H at δ 7.44 and 6-H at 7.02; 9-C at δ 149.9 showed no correlation to 6-H. The resulting 8-(*N*-alkylaminomethyl)coumarins **(2)** were then coupled with bis-β-chloroethyl dichlorophosphamide to form a series of coumarin-7,8-cyclophosphoramide analogs **(3)** (Table 1).

In conclusion, a convenient procedure for preparation of novel coumarin-7,8-cyclophosphoramide analogs were reported using commercially available materials. These compounds were synthesized by two steps: Mannich reaction and cyclized reaction. Mannich reaction, under the present experimental conditions, was found to proceed regioselectively, favoring attack at 8-C of 7-hydroxycoumarin. The synthetic procedure of coumarin-7,8-cyclophosphoramide analogs might be valuable for development of new cyclophosphamide coumarin prodrugs.

EXPERIMENTAL

All starting materials were obtained from commercial sources and used without further purification. Melting points were recorded on a microscopical determinator XT4 (the thermometer was not adjusted). IR spectra were recorded on a Shimadazuir-408 spectrophotomer. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra were obtained on a Bruker Avance DPX-400 spectrophotomer; chemical shifts were expressed in parts per million with positive values downfield from internal tetramethylsilane (TMS) and external 85% H_3PO_4 (^{31}P). Coupling constants were expressed in Hertz. ESI-MS was recorded on a Bruker Esquire-3000 instrument.

7-Hydroxycoumarin **(1)** (5 mmol) was dissolved in THF (20 ml), and the appropriate amine (5 mmol) and 37% formaldehyde (5 mmol) were added. The resulting mixture was refluxed for 8–12 h, and the reaction was monitored by thin-layer chromatography (TLC). After cooling at −5 to 10 °C, it was left overnight, and the residue was filtered. The crude Mannich base **(2)** was obtained as a yellow solid, which was used for the following reaction without further purification.

A solution of bis-*β*-chloroethyl dichlorophosphamide (2 mmol)[15] in dry THF (10 mL) was added dropwise into a stirred solution of crude Mannich base **(2)**

(2 mmol) and triethylamine (4 mmol) in dry THF (20 ml) at room temperature over 30 min. The resulting mixture was further stirred for 2 h. The temperature was slowly raised to 50–55 °C and maintained at this temperature for 3–4 h. After the triethylamine hydrochloride was filtered off, the solution was evaporated under reduced pressure to afford a white solid residue. It was further purified by silica-gel column chromatography, eluting with methanol and ethyl acetate (1:5, v/v).

Compound 3a. Yellow powder, mp 117–118 °C; ^{1}H NMR ($CDCl_3$ 400 MHz) δ: 0.97 (m, 3H, $-CH_2CH_2CH_3$), 1.80–1.70 (m, 2H, $-CH_2CH_2CH_3$), 3.18–3.09 (m, 2H, $-CH_2CH_2CH_3$), 3.71–3.36 (m, 8H, 15-H & 16-H), 4.54–4.50 (m, 2H, 14-H), 6.37 (d, $J = 9.56$, 1H, 3-H), 6.96 (d, $J = 8.52$, 1H, 6-H), 7.42 (d, $J = 8.52$, 1H, 5-H), 7.71 (d, $J = 9.60$, 1H, 4-H). ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 11.3 ($-CH_2CH_2CH_3$), 21.1 (d, $-CH_2CH_2CH_3$, $J = 3.18$), 42.2 (14-C), 43.5 (16-C), 49.3 (d, $-CH_2CH_2CH_3$, $J = 4.36$), 50.0 (d, 15-C, $J = 2.8$), 111.6 (d, 6-C, $J = 7.21$), 114.6 (3-C), 114.7 (10-C), 115.0 (d, 8-C, $J = 7.34$), 128.0 (5-C), 143.5 (4-C), 150.6 (9-C), 153.3 (d, 7-C, $J = 8.42$), 159.9 (2-C); ^{31}P NMR ($CDCl_3$ 162 MHz) δ: 7.99; IR (KBr): 1236 cm^{-1} (P=O); ESI-MS, m/z: 441 $[M + Na]^+$; HR-MS (ESI): m/z $[M + Na]^+$ calcd. for $C_{17}H_{21}Cl_2N_2O_4P$: 441.0514; found: 441.00519.

Compound 3b. Yellow powder, mp 137–138 °C; ^{1}H NMR ($CDCl_3$ 400 MHz) δ: 1.31 [m, 6H, $-CH(CH_3)_2$], 3.71–3.36 (m, 8H, 15-H & 16-H), 4.00–3.95 [m, 1H, $-CH(CH_3)_2$], 4.46–4.42 (m, 2H, 14-H), 6.37 (d, $J = 9.60$, 1H, 3-H), 6.96 (d, $J = 8.52$, 1H, 6-H), 7.43 (d, $J = 8.52$, 1H, 5-H), 7.73 (d, $J = 9.56$, 1H, 4-H). ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 20.3 [$-CH(CH_3)_2$], 35.9 (14-C), 42.1 (16-C), 46.9 [d, $J = 2.86$, $-CH(CH_3)_2$], 49.2 (d, $J = 4.06$, 15-C), 112.2 (d, 6-C, $J = 7.16$), 114.7 (3-C), 114.8 (10-C), 114.9 (d, 8-C, $J = 7.16$), 128.0 (5-C), 143.5 (4-C), 150.6 (9-C), 153.5 (d, 7-C, $J = 8.55$), 160.0 (2-C); ^{31}P NMR ($CDCl_3$ 162 MHz) δ: 8.69; IR (KBr): 1248 cm^{-1} (P=O); ESI-MS, m/z: 441 $[M + Na]^+$; HR-MS (ESI): m/z $[M + Na]^+$ calcd. for $C_{17}H_{21}Cl_2N_2O_4P$: 441.0514; found: 441.0518.

Compound 3c. Yellow powder, mp 143–144 °C; ^{1}H NMR ($CDCl_3$ 400 MHz) δ: 0.98 (m, 3H, $-CH_2CH_2CH_2CH_3$), 1.41–1.35 (m, 2H, $-CH_2CH_2CH_2CH_3$), 1.76–1.67 (m, 2H, $-CH_2CH_2CH_2CH_3$), 3.20–3.12 (m, 2H, $-CH_2CH_2CH_2CH_3$), 3.71–3.37 (m, 8H, 15-H & 16-H), 4.53–4.49 (m, 2H, 14-H), 6.36 (d, $J = 9.56$, 1H, 3-H), 6.95 (d, $J = 8.52$, 1H, 6-H), 7.43 (d, $J = 8.52$, 1H, 5-H), 7.73 (d, $J = 9.60$, 1H, 4-H); ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 13.8 ($-CH_2CH_2CH_2CH_3$), 20.1 ($-CH_2CH_2CH_2CH_3$), 29.8 ($-CH_2CH_2CH_2CH_3$), 42.1 (14-C), 43.4 (16-C), 48.1 ($-CH_2CH_2CH_2CH_3$), 49.2 (d, 15-C, $J = 4.00$), 111.4 (6-C), 111.5 (3-C), 114.7 (10-C), 114.9 (d, 8-C, $J = 7.21$), 128.0 (5-C), 143.5 (4-C), 150.6 (9-C), 153.3 (d, 7-C, $J = 8.40$), 159.9 (2-C); ^{31}P NMR ($CDCl_3$ 162 MHz): δ: 8.60; IR (KBr): 1244 cm^{-1} (P=O); ESI-MS, m/z: 455 $[M + Na]^+$; HR-MS (ESI): m/z $[M + Na]^+$ calcd. for $C_{18}H_{23}Cl_2N_2O_4P$: 455.0677; found: 455.0670.

Compound 3d. Yellow powder, mp 132–133 °C; ^{1}H NMR ($CDCl_3$ 400 MHz) δ: 1.47 [s, 9H, $-C(CH_3)_3$], 3.53–3.39 (m, 4H, 15-H), 3.73–3.59 (m, 4H, 16-H), 4.68–4.29 (m, 2H, 14-H), 6.38 (d, $J = 9.56$, 1H, 3-H), 7.02 (d, $J = 8.40$, 1H, 6-H), 7.44 (d, $J = 8.44$, 1H, 5-H), 7.72 (d, $J = 9.60$, 1H, 4-H); ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 28.7 [$-C(CH_3)_3$], 39.1 (d, 14-C, $J = 1.90$), 42.0 (16-C), 50.2 (d, 15-C,

$J = 4.17$), 56.9 [d, $-\underline{C}(CH_3)_3$, $J = 3.83$], 114.9 (3-C), 114.9 (d, 6-C, $J = 5.6$), 115.2 (10-C), 115.3 (d, 8-$\overline{C}$, $J = 7.20$), 128.3 (5-C), 143.6 (4-C), 149.9 (9-C), 153.8 (d, 7-C, $J = 8.44$), 159.9 (2-C); ^{31}P NMR ($CDCl_3$ 162 MHz); δ: 8.62; IR (KBr): 1259 cm^{-1} (P=O); ESI-MS, m/z: 455 $[M + Na]^+$; HR-MS (ESI): m/z $[M + Na]^+$ calcd. for $C_{18}H_{23}Cl_2N_2O_4P$: 455.0677; found: 455.0672.

Compound 3e. Yellow powder, mp 179–181 °C; ^{1}H NMR ($CDCl_3$ 400 MHz) δ: 1.98–1.13 & 3.45–3.36 (m, 11H, cyclohexyl-H), 3.72–3.50 (m, 8H, 15-H & 16-H), 4.47 (m, 2H, 14-H), 6.37 (d, $J = 9.56$, 1H, 3-H), 6.95 (d, $J = 8.52$, 1H, 6-H), 7.41 (d, $J = 8.48$, 1H, 5-H), 7.71 (d, $J = 9.56$, 1H, 4-H); ^{13}C NMR ($CDCl_3$ 100 MHz) δ: 25.2, 25.8, 30.6, 55.3 (cyclohexane), 37.2 (14-C), 42.2 (16-C), 49.2 (d, 15-C, $J = 4.36$), 112.5 (d, 6-C, $J = 7.00$), 114.7 (3-C), 114.7 (10-C), 115.0 (d, 8-C, $J = 7.19$), 128.0 (5-C), 143.5 (4-C), 150.6 (9-C), 153.6 (d, 7-C, $J = 8.44$), 160.1 (2-C); ^{31}P NMR ($CDCl_3$ 162 MHz) δ: 8.87; IR (KBr): 1247 cm^{-1} (P=O); ESI-MS, m/z: 481 $[M + Na]^+$; HR-MS (ESI): m/z $[M + Na]^+$ calcd. for $C_{20}H_{25}Cl_2N_2O_4P$: 481.0827; found: 481.0821.

ACKNOWLEDGMENTS

We are grateful to the National Science Foundation of China (No. 20472076) and the Henan Science Foundation for Excellent Youth (No. 0512001400) for financial support.

REFERENCES

1. Cox, D.; O'Kennedy, R.; Thornes, R. D. The rarity of liver toxicity in patients treated with coumarin (1,2-benzopyrone). *Hum. Toxicol.* **1989**, *8*, 501.
2. Soine, T. O. Naturally occurring coumarins and related physiological activities. *J. Pharm. Sci.* **1964**, *53*, 231.
3. Jurd, L.; King Jr., A. D.; Mihara, K. Antimicrobial properties of umbelliferone derivatives. *Phytochemistry* **1971**, *10*, 2965.
4. Mazzei, M.; Dondero, R.; Sottofattori, E.; Melloni, E.; Minafra, R. Inhibition of neutrophil O_2^- production by unsymmetrical methylene derivatives of benzopyrans: Their use as potential anti-inflammatory agents. *Eur. J. Med. Chem.* **2001**, *36*, 851.
5. Sardari, S.; Mori, Y.; Horita, K.; Micetich, R. G.; Nishibe, S.; Daneshtalab, M. Synthesis and antifungal activity of coumarins and angular furanocoumarins. *Bioorg. Med. Chem.* **1999**, *7*, 1933.
6. Kasinadhuni, V. R. R.; Rajashekhar, G.; Rajagopalan, R.; Sharma, V. M.; Krishna, C. V.; Sairam, P.; Prasad, G. S.; Sadhukhan, S.; Rao, G. G. Anti-ulcer potential of Haldinia cordifolia. *Fitoterapia* **1999**, *70*, 93.
7. Marshall, M. E.; Mendelsohn, L.; Butler, K.; Riley, L.; Cantrell, J.; Wiseman, C.; Taylor, R.; Macdonald, J. S. Treatment of metastatic renal cell carcinoma with coumarin (1,2-benzopyrone) and cimetidine: A pilot study. *J. Clin. Oncol.* **1987**, *5*, 862.
8. Marshall, M. E.; Butler, K.; Hermansen, D. Treatment of hormone-refractory stage D carcinoma of prostate with coumarin (1,2-benzopyrone) and cimetidine: A pilot study. *Prostate* **1990**, *17*, 95.
9. Mohler, J. L.; Gomella, L. G.; Crawford, E. D.; Glode, L. M.; Zippe, C. D.; Fair, W. R.; Marshall, M. E. Phase II evaluation of coumarin (1,2-benzopyrone) in metastatic prostatic carcinoma. *Prostate* **1992**, *20*, 123.

10. Thornes, R. D.; Daly, L.; Lynch, G.; Breslin, B.; Browne, H.; Browne, H. Y.; Corrigan, T.; Daly, P.; Edwards, G.; Gaffney, E. Irish melanoma group: Treatment with coumarin to prevent or delay recurrence of malignant melanoma. *J. Cancer Res. Clin. Oncol.* **1994**, *120*(Suppl.), 32.
11. Egan, D.; O'Kennedy, R.; Moran, E.; Cox, D.; Prosser, E.; Thornes, R. D. The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds. *Drug Metab. Rev.* **1990**, *22*, 503.
12. Marshall, M. E.; Kervin, K.; Benefield, C.; Umerani, A.; Albainy-Jenei, S.; Zhao, Q.; Khazaeli, M. B. Growth-inhibitory effects of coumarin (1,2-benzopyrone) and 7-hydroxycoumarin on human malignant cell lines in vitro. *J. Cancer Res. Clin. Oncol.* **1994**, *120*(Suppl.), 3.
13. Jimenez-Orozco, F. A.; Lopez-Gonzalez, J. S.; Nieto-Rodriguez, A. Decrease of cyclin D1 in the human lung adenocarcinoma cell line A-427 by 7-hydroxycoumarin. *Lung Cancer* **2001**, *34*, 185.
14. Li, Z.; Han, J.; Jiang, Y.; Browne, P.; Knox, R. J.; Hu, L. Nitrobenzocyclophosphamides as potential prodrugs for bioreductive activation: Synthesis, stability, enzymatic reduction, and antiproliferative activity in cell culture. *Bioorg. Med. Chem.* **2003**, *11*, 4171.
15. Stec, W. J. Cyclophosphamide and its congeners. *Organophosphorous Chem.* **1982**, *13*, 145.