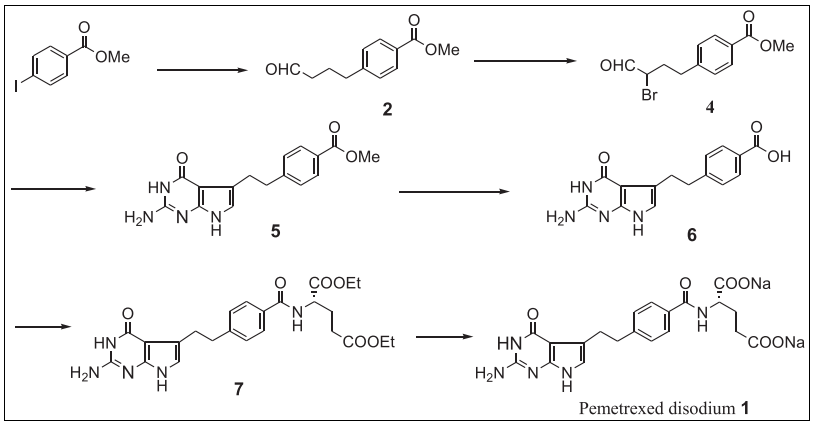
An Efficient Synthesis of Pemetrexed Disodium

H. Qi,[a] J. Wen,[a] L. Li,[a] R. Bai,[a] L. Chen,[a]* and D. Wang[b]

[a]School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, People's Republic of China
[b]School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, People's Republic of China
*E-mail: lgchen@tju.edu.cn

Received September 21, 2013
DOI 10.1002/jhet.2164
Published online 00 Month 2014 in Wiley Online Library (wileyonlinelibrary.com).



An efficient synthetic method for the pemetrexed disodium has been developed using methyl 4-iodobenzoate and 3-buten-1-ol as starting materials via six steps. The developed process avoided some tedious workup procedures and unfriendly reagents compared with the reported synthetic routes. In addition, two impurities generated in the process were isolated and characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. The mechanisms of the two impurities were also discussed, and the impurities could be easily removed by suitable workup procedures. The overall yield of pemetrexed disodium was increased from 12.8% (literature) to 34.9%. Therefore, this cost-effective, environmental friendly, and high-yielding process is more suitable for scale-up production of pemetrexed disodium.

J. Heterocyclic Chem., **00,** 00 (2014).

INTRODUCTION

Pemetrexed disodium (tradename Alimta; N-[4-[2-(2-amino-3,4-dihydro-4-oxo-7*H*-pyrrolo-[2,3-*d*]pyrimidin-5-yl) ethyl]-benzoyl]-L-glutamic acid disodium), a new-generation multi-targeted antifolate that inhibits thymidylate synthase, dihydrofolate reductase, and glycinamide ribonucleotide formyl transferase, plays crucial roles in pyrimidine and purine synthesis, resulting in the inhibition of DNA and RNA synthesis [1]. This agent is, at the moment, approved in the USA and Europe for the treatment of advanced mesothelioma in combination with cisplatin and for advanced nonsmall cell lung cancer, in first line, associated with cisplatin and, in second line, as single agent [2]. Furthermore, plenty of clinical studies have shown that pemetrexed is active in other solid tumors, such as breast cancer, endometrial carcinoma, and nasopharyngeal carcinoma [3–5].

Pemetrexed disodium (Figure 1) was first synthesized by Taylor and his co-workers at Princeton University [6], and then, a number of synthetic methods have been reported for the preparation of pemetrexed during recent years [7–12]. Among them, the most applicable synthetic method [8] was shown in Scheme 1. Pemetrexed disodium was prepared in 10 steps from methyl 4-bromobenzoate. However, this process suffered from several drawbacks such as the following: (i) tedious workup procedures in some steps, for example, purification of **2** by converting it into its bisulfate **3** with $NaHSO_3$ and purification of **7** by converting it into its tosilate **8** with p-toluenesulfonic acid; (ii) usage of unfriendly reagents such as $(CH_3)_3SiCl$ making the process industrially unattractive; and (iii) low total yield (less than 13%). Thus, this synthetic route was modified in this paper; a more efficient and higher-yielding synthesis (Scheme 2) of pemetrexed disodium with an overall yield of 34.9% was summarized and reported here.

RESULTS AND DISCUSSION

In Scheme 1, **2** was purified by converting it into its bisulfate adduct with $NaHSO_3$ and then regenerating of the aldehyde with $(CH_3)_3SiCl$. This is unsuitable for the industrial production due to using of toxic and flammable $(CH_3)_3SiCl$ as reagent and low yield. In order to simplify workup procedures and improve the yield, a simple method for

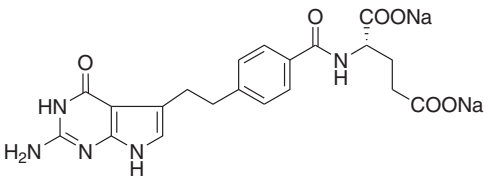
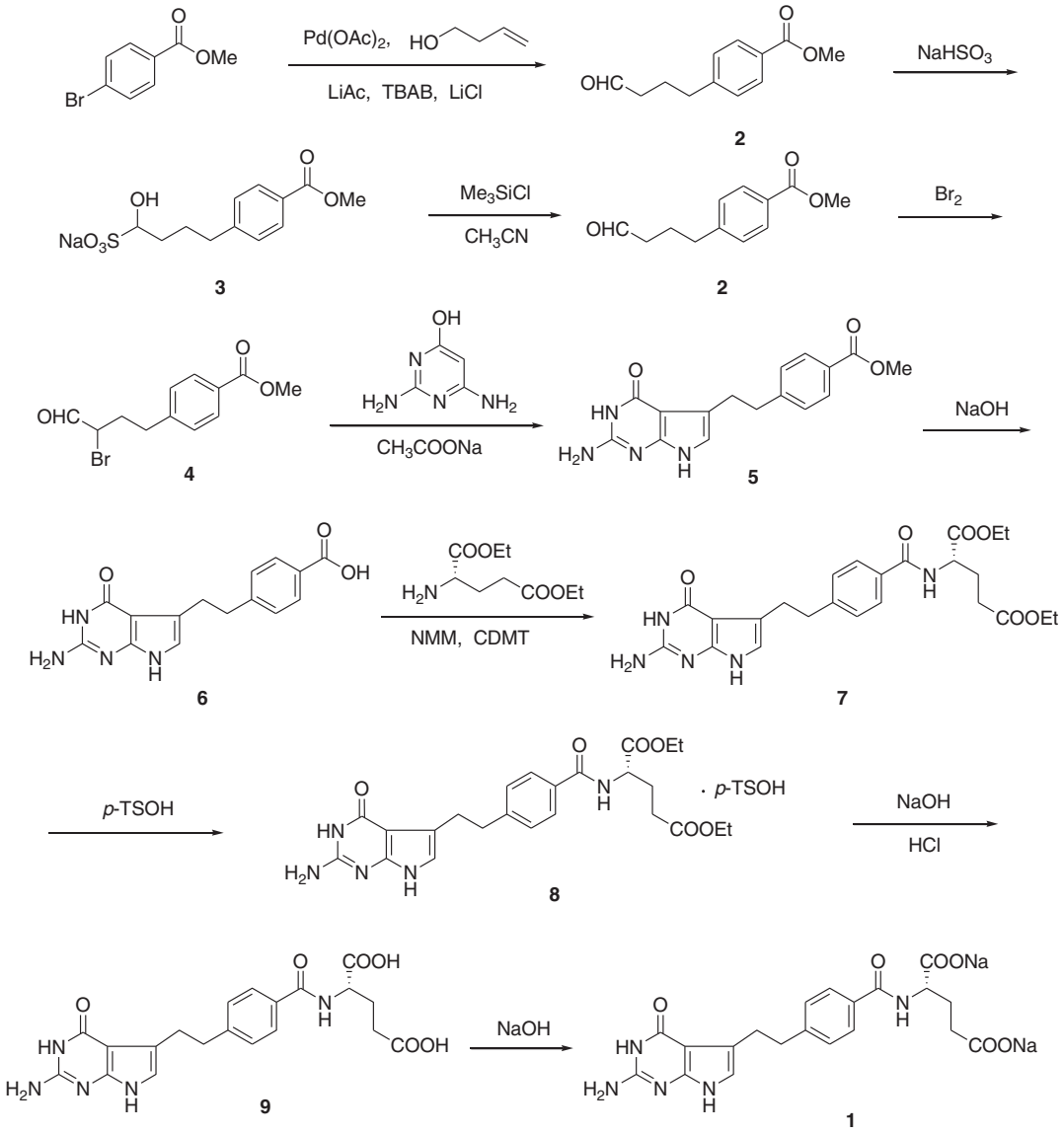


Figure 1. Chemical structure of pemetrexed disodium.

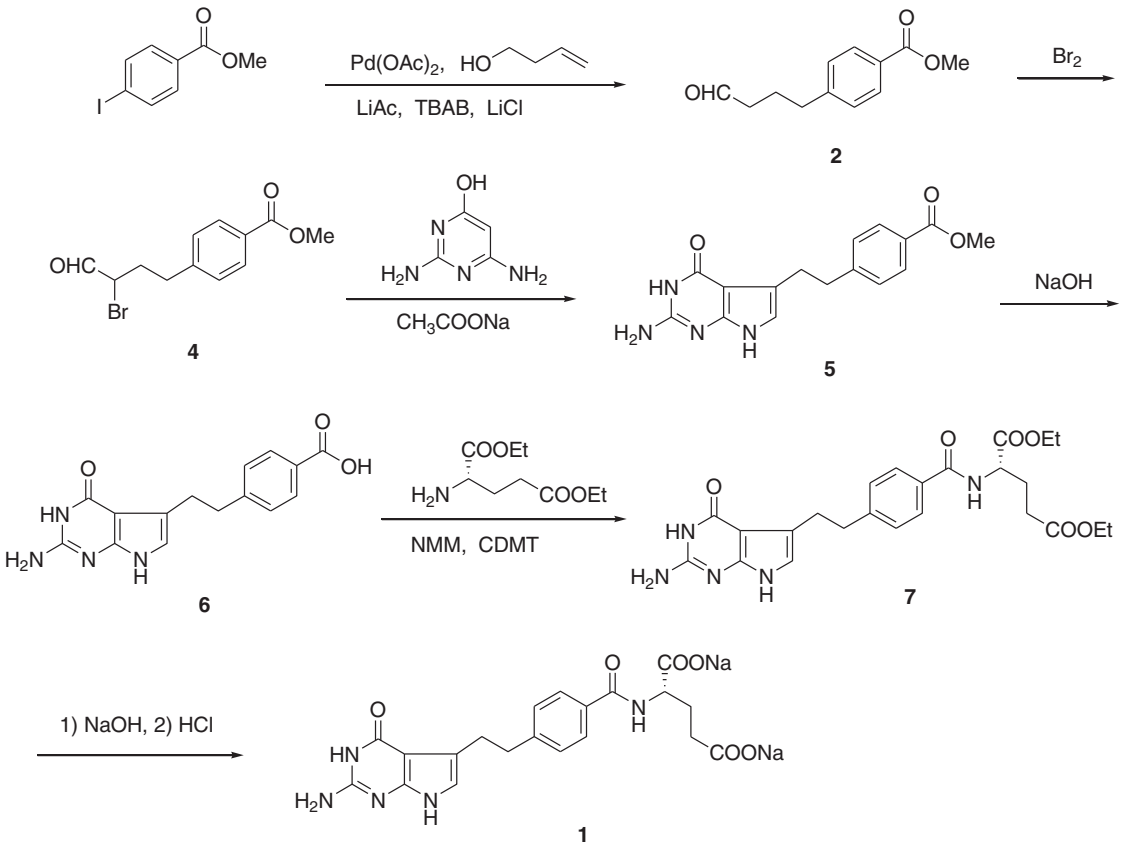
purification of **2** was applied (Scheme 2). After the reaction was completed, **2** of high purity could be directly obtained through extraction with ethyl acetate, decoloration with activated carbon and silica gel, instead of treatment of crude **2** with $NaHSO_3$ and $(CH_3)_3SiCl$. The intermediate **2** could be used directly to the next bromination step without further purification.

During the posttreatment of bromination reaction to get compound **4** (Scheme 2), a byproduct (3.1% of the total) was isolated, which was confirmed by ^1H NMR, ^{13}C NMR, and HRMS as compound **10**. We speculated that the byproduct **10** was generated via oxidation of the aldehyde **2** by hypobromous acid in the presence of Br_2 and H_2O (Scheme 3). However, considering that the byproduct **10** has no negative effect on the following cyclization and can be easily removed in the workup procedure of the cyclization, the crude product **4** could be used directly to the next cyclization step without further purification.

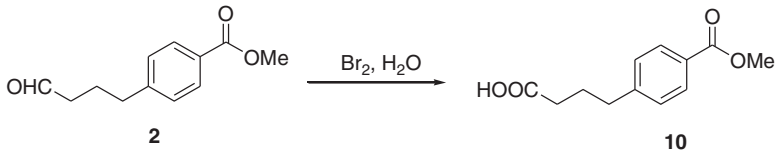
Scheme 1. Reported synthetic scheme of pemetrexed disodium.



Scheme 2. Modified synthetic scheme of pemetrexed disodium.



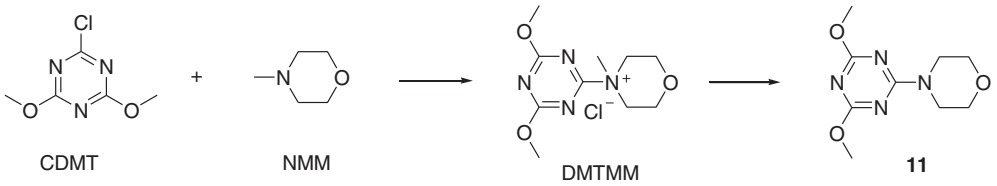
Scheme 3. A possible mechanism for formation of byproduct 10.



During the purification of the coupling reaction to afford **7** (Scheme 2), another byproduct (4.0% of the total) was also isolated, which was confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS as compound **11**. A possible mechanism for the formation of byproduct **11** was proposed as shown in Scheme 4: N-Methylmorpholine was treated with 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) via a salification reaction in N,N-dimethylformamide (DMF) to give4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride, which partially decomposed into **11** by demethylation. Obviously, it is difficult to prevent the formation of the byproduct **11** in this reaction. Through further investigation, we found that the byproduct **11** could be easily removed through recrystallization in petroleum ether–ethyl acetate (3:1). In addition, the compound **7** of high purity was efficiently obtained in this way, instead of treatment of crude **7** with p-toluenesulfonic acid.

In Scheme 1, pemetrexed disodium was prepared in two steps from **8**. In the first step, **8** was hydrolyzed by the treatment with NaOH and then acidified with HCl to afford pemetrexed acid (**9**); in the second step, the disodium salt was obtained via neutralization of **9** with NaOH. In order to simplify the postprocessing steps and improve the yield, a convenient method for synthesis of pemetrexed disodium starting from intermediate **7** (Scheme 2) was applied. Without separation of pemetrexed acid, crude pemetrexed disodium was obtained directly with NaOH and ethanol by one-pot reaction. In addition, we also found a good way for purification of the target product, the high quality pemetrexed disodium can be obtained by recrystallization of crude pemetrexed disodium in H_2O-EtOH (1:3).

Scheme 4. A possible mechanism for formation of byproduct 11.



CONCLUSION

In conclusion, we have developed a cost-effective, environmental friendly, and high-yielding synthetic route of pemetrexed disodium. All intermediates and the disodium salt could be prepared with readily available, inexpensive, and environmentally friendly reagents via simple workups, instead of tedious workup procedures of the previous methods. In addition, two impurities generated in the process were isolated, confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS, and their formation mechanism were proposed. Pemetrexed disodium was obtained in 34.9% total yield and 99.9% purity. Therefore, the synthetic route is more suitable for large-scale industrial production.

EXPERIMENTAL

Reagents and solvents were obtained from commercial suppliers. All reactions were monitored by thin layer chromatography using commercial silica gel plates. The purity of products was detected by HPLC on Agilent 1100 series. Melting points were observed on YRT-3 Melting Point Tester (Precision Instrument Factory of Tianjin University, Tianjin, China) and were uncorrected. The IR spectra were recorded on a Bruker model TENSOR 27 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) for KBr disc. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE III 400 MHz. HRMS was recorded on MicrOTOF-Q II (Bucker Daltonics Inc., Billerica, MA).

4-(4-Carbomethoxyphenyl)butanal(2). Methyl 4-iodobenzoate (15.00 g, 57 mmol) was dissolved in 220 mL of N,N-dimethylformamide (DMF). To the solution were added anhydrous lithium acetate (6.43 g, 63 mmol), lithium chloride (7.21 g, 171 mmol), and tetrabutyl ammonium bromide (8.67 g, 27 mmol). Nitrogen was bubbled through for 5 min. Then, 3-buten-1-ol (4.95 g, 68 mmol) and palladium acetate (0.32 g, 1.4 mmol) were added. The reaction mixture was stirred at 60°C for 10 h. After cooling to room temperature, 300 mL of water was added and stirred for 15 min, and then, the resulting mixture was extracted with ethyl acetate (3 × 100 mL), the organic layer was added 15 g of activated carbon and 15 g of silica gel and then stirred for half an hour to be decolorized. After filtration, the solvent was evaporated under reduced pressure to afford the compound **2** (10.70 g, 91.2% yield) as yellow oil (lit.[7] light yellow oil) with a purity of 96.0%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.95–2.03 (m, 2H), 2.48 (t, J = 7.2 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 3.90 (s, 3H), 7.26 (d, J = 8.0 Hz, 2H), 7.99 (d, J = 8.0 Hz, 2H), 9.78 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.25, 34.98, 43.02, 51.99, 128.18, 128.47, 129.82, 146.71, 167.0, 201.8. IR (KBr. cm^{-1}) 3428 (m), 2973 (m), 1722 (s), 1610 (m), 1436 (m), 1377 (w), 1281 (s), 1180 (m), 1109 (m), 880 (w), 800 (m), 706 (w).

Methyl 4-(3-bromo-4-oxobutyl) benzoat e(4). The aforementioned product **2** (10.70 g, 52 mmol) was added to acetonitrile (100 mL). The solution was cooled to 0°C with an ice bath, and Br_2 (8.32 g, 52 mmol) was added dropwise to it. After the addition, the ice bath was removed, and the reaction mixture was stirred at room temperature for 2 h; 50 mL deionized water was added and stirred for 15 min. After that, the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL), the organic phases were combined and dried with sodium sulfate and then concentrated under reduced pressure to give a light yellow oil (lit.[12] yellow oil) **4** (12.40 g, 84.3% yield) with a purity of 93.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.19 ~ 2.26(m, 1H), 2.35 ~ 2.41(m, 1H), 2.79 ~ 2.84 (m, 1H), 2.91 ~ 2.95 (m, 1H), 3.90 (s, 3H), 4.17 ~ 4.19 (m, 1H), 7.27 (d, J = 7.8 Hz, 2H), 7.97 (d, J = 7.8 Hz, 2H), 9.46 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 32.62, 32.77, 52.07, 54.37, 128.6, 129.8, 130.0, 145.1, 166.9, 192.4. IR (KBr. cm^{-1}) 3465 (w), 2951 (w), 1720 (s), 1609 (m), 1435 (m), 1370 (w), 1280 (s), 1179 (m), 1058 (m), 1020 (w), 764 (w), 706 (w).

Methyl 4-[2-(2-amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl)ethyl]benzoate(5). The intermediate was prepared according to the literature [12]. Yield: 89.0%. mp > 250°C (lit.[7] mp > 250°C). ^{1}H NMR (400 MHz, DMSO−d_6) δ: 2.86 (t, J = 8.4 Hz, 2H), 3.00 (t, J = 8.4 Hz, 2H), 3.83 (s, 3H), 6.03 (s, 2H), 6.32 (s, 1H), 7.34 (d, J = 8.0 Hz, 2H), 7.86 (d, J = 8.0 Hz, 2H), 10.2 (s, 1H), 10.6 (s, 1H). ^{13}C NMR (100 MHz, DMSO−d_6) δ: 28.31, 36.75, 52.40, 99.18, 113.9, 118.0, 127.5, 129.2, 129.6, 148.8, 151.8, 152.7, 159.8, 166.7. IR (KBr. cm^{-1}) 3481 (w), 2951 (w), 1720 (s), 1609 (m), 1435 (m), 1370 (w), 1280 (s), 1233 (w), 1069 (w), 864 (w).

4-[2-(2-Amino-4,7-dihydro-4-oxo-1*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl)ethyl]benzoic-acid(6). The intermediate was prepared according to the literature [12]. Yield: 93.1%. mp > 260°C (lit.[7] mp > 250°C). ^{1}H NMR (400 MHz, DMSO−d_6) δ: 2.86 (t, J = 7.6 Hz, 2H), 2.99 (t, J = 7.6 Hz, 2H), 6.37 (s, 1H), 6.42 (br, 1H), 7.31 (d, J = 7.6 Hz, 2H), 7.84 (d, J = 8.0 Hz, 2H), 10.50 (br, 1H), 10.80 (s, 1H). ^{13}C NMR (100 MHz, DMSO−d_6) δ: 27.98, 36.53, 99.29, 114.7, 118.7, 128.7, 129.0, 129.8, 148.0, 152.1, 158.7, 167.8. IR (KBr. cm^{-1}) 3475 (m), 3206 (m), 2922 (m), 1663 (s), 1447 (w), 1387 (m), 1310 (m), 1178 (w), 1054 (w), 866 (w).

***N*-[4-(2-Amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid diethyl eater(7)**. Compound **6** (8.64 g, 29 mmol) was suspended in DMF (100 mL). The mixture was stirred for 15 min at room temperature; then, N-methylmorpholine (8.73 g, 86 mmol) and CDMT (6.70 g, 38 mmol) were added with an ice/water bath under nitrogen atmosphere. Then, the ice/water bath was removed, and the reaction mixture was stirred at room temperature for 1 h, diethyl L-glutamate hydrochloride (9.13 g, 38 mmol) was added and the reaction mixture was further stirred for 2 h at 35°C. After the reaction was

completed, to the reaction mixture was added dichloromethane (80 mL) and deionized water (80 mL), stirred for 15 min, and then, the resulting mixture was allowed to separate, the aqueous layer was extracted with dichloromethane (50 mL), the combined organic layers were evaporated to give a yellow viscous oil, which was added with petroleum ether (90 mL) and ethyl acetate (30 mL), stirred for 30 min at 40°C, and a large amount of light yellow solid appeared; the solid was filtered at 40°C and dried to give **7** (11.92 g, 85.5% yield). mp: 168–170°C (lit.[7] mp: 169–171°C). ^{1}H NMR (400 MHz, DMSO−d_6) δ: 1.15 ~ 1.20 (m, 6H),1.97 ~ 2.13 (m, 4H), 2.46 (t, J = 7.6 Hz, 2H), 2.68 ~ 2.78 (m, 2H), 4.00 ~ 4.13 (m, 4H), 4.40 ~ 4.45 (m, 1H), 6.73 (br, 1H), 7.29 (d, J = 8.0 Hz, 2H), 7.79 (d, J = 8.0 Hz, 2H), 8.66 (d, J = 8.0 Hz, 1H),10.42 (s, 1H), 10.61 (s, 1H). ^{13}C NMR (100 MHz, DMSO−d_6) δ: 14.03, 25.69, 27.97, 30.15, 36.11, 51.94, 54.87, 59.89, 60.50, 98.70, 113.40, 117.60, 127.37, 128.16, 131.07, 146.28, 151.31, 152.18, 159.28, 166.63, 171.82, 172.19. IR (KBr. cm^{-1}) 3337 (m), 3209 (m), 2981 (w), 1736 (s), 1664 (s), 1522 (m), 1432 (m), 1374 (m), 1259 (w), 1019 (w), 854 (w), 774 (m).

***N*-[4-[2-(2-Amino-3,4-dihydro-4-oxo-7*H*-pyrrolo[2,3-*d*] pyrimidin-5-yl)ethyl]-benzoyl]-L-glutamic acid disodium salt(pemetrexed disodium, 1)**. Intermediate **7** (10.00 g, 20 mmol) was added to a 2 N aqueous sodium hydroxide solution (100 mL). The mixture was stirred for 2 h at room temperature, and then, the pH of the solution was adjusted to 7.5 ~ 8.5 using 1 N hydrochloric acid, the solution was stirred for 30 min at room temperature, ethanol (100 mL) was added and further stirred for 10 min, then the solution was kept in refrigerator over night, and a large amount of off-white solid appeared. After filtration, the solid was added ethanol (120 mL) and deionized water (40 mL), stirred for 30 min at room temperature, then the solution was kept in refrigerator over night again, and white crystal appeared, the crystal was filtered and dried to give **1** (6.10 g, 65.1% yield) with a purity of 99.9%. mp > 260°C (lit.[7] mp > 250°C). ^{1}H NMR (400 MHz, D_2O) δ: 1.90 ~ 1.96 (m, 1H), 2.04 ~ 2.10 (m, 1H), 2.20 ~ 2.22 (m, 2H), 2.81 (m, 4H), 4.21 ~ 4.23 (m, 1H), 6.28 (s, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H). ^{13}C NMR (100 MHz, D_2O) δ: 26.78, 28.45, 34.34, 35.48, 56.0, 98.81, 115.44, 118.09, 127.0, 128.49, 130.68, 146.7, 150.8, 152.25, 161.42, 170.03, 179.04, 182.34. IR (KBr. cm^{-1}) 3017 (w), 2954 (m), 2870 (w), 1733 (s), 1619 (s), 1609 (m), 1429 (m), 1274 (s), 1202 (m), 1111 (m), 1017 (w), 936 (w), 767 (m), 705 (m). HRMS (ESI), calcd: $C_{20}H_{19}N_5Na_2O_6$ $[M+H]^+$ *m/z*: 472.1131, found:472.1206.

4-(4-(Methoxycarbonyl)phenyl)butanoicacid (10). Melting point: 76–78°C (lit.[13] mp: 76.5–78°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.96 ~ 2.00 (m, 2H), 2.38 (t, J = 7.2 Hz, 2H), 2.73 (t, J = 7.8 Hz, 2H), 3.90 (s, 3H), 7.25 (d, J = 7.8 Hz, 2H), 7.96 (d, J = 8.4 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.83, 33.23, 34.98, 52.04, 128.1, 128.5, 129.8, 146.7, 167.1, 179.5. IR (KBr. cm^{-1}) 3428 (m), 2919 (m), 2850 (w), 1721 (s), 1607 (m), 1559 (m), 1436 (m), 1282 (s), 1110 (s), 797 (w), 699 (w). HRMS (ESI), calcd: $C_{12}H_{14}O_4$, $[M-H]^+$ *m/z*: 221.0892, found:221.0824.

2,4-Dimethoxy-6-morpholino-1,3,5-triazine (11). Melting point: 128–130°C (lit.[14] mp: 129–130°C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.73 (t, J = 4.8 Hz, 4H), 3.85 (t, J = 4.2 Hz, 4H), 3.96 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 43.96, 54.52, 66.60, 166.8, 172.3. IR (KBr. cm^{-1}) 1580 (s), 1541 (s), 1468 (s), 1365 (m), 1306 (w), 1252 (m), 1208 (m), 1128 (m), 1071 (w), 1006 (m), 875 (m), 810 (m). HRMS (ESI), calcd: $C_9H_{14}N_4O_3$ $[M+H]^+$ *m/z*: 227.1066, found: 227.1136.

REFERENCES AND NOTES

[1] Ochi, N.; Yamane, H.; Yamagishi, T.; Takigawa, N. Lung Cancer 2013, 80, 111.

[2] Tiseo, M.; Gelsomino, F.; Bartolotti, M.; Brili, M. P.; Ardizzoni, A. Lung Cancer 2009, 65, 251.

[3] Yau, T. K.; Shum, T.; Lee, A. W. M.; Yeung, M. W.; Ng, W. T., Chan, L. Oral Oncol 2012, 48, 441.

[4] Miller, D. S.; Blessing, J. A.; Drake, R. D.; Higgins, R.; McMeekin, D. S.; Puneky, L. V.; Krasner, C. N. Gynecol Oncol 2009, 115, 443.

[5] Pippen, J.; Elias, A. D.; Neubauer, M.; Stokoe, C.; Vaughn, L. G.; Wang, Y.; Orlando, M.; Shonukan, O.; Muscato, J.; O'Shaughnessy, J. A.; Gralow, J. Clin Breast Cancer 2010, 10, 148.

[6] Taylor, E. C.; Kuhnt, D. J Med Chem 1992, 35, 4450.

[7] Barnett, C. J.; Wilson, T. M.; Kobierski, M. E. Org Proc Res Dev 1999, 3, 184.

[8] Kjell, D. P.; Slattery, B. J.; Barnett, C. J. U.S. Patent 6,013,828, 2000; Chem Abstr 2000, 132, 64061x.

[9] Taylor, E. C.; Liu, B. J Org Chem 2001, 66, 3726.

[10] Taylor, E. C.; Liu, B. J Org Chem 2003, 68, 9938.

[11] Jens, A. U.; Hannes, H.; Valiervich, N. V. WO/2011/064256 A1, 2011; Chem Abstr 2011, 154, 623999.

[12] Li, J.; Zhao, N. Eur Patent 2,301,909 A1, 2011; Chem Abstr 2009, 152, 12373.

[13] Gangjee, A.; Zeng, Y.; McGuire, J. J.; Mehraein, F.; Kisliuk, R. L. J Med Chem 2004, 47, 6893.

[14] Kunishima, M.; Kawachi, C.; Iwasaki, F.; Terao, K.; Tani, S. Tetrahedron Lett 1999, 40, 5327.