Chemistry of Heterocyclic Compounds, Vol. 36, No. 7, 2000

SYNTHESIS OF METHYL ESTERS OF 5-AMINO-4-(SUBSTITUTED AMINO)-2-METHYLTHIO-7H-PYRROLO[2,3-*d*]-PYRIMIDINE-6-CARBOXYLIC ACIDS

S. Tumkevicius, A. Urbonas, and P. Vainilavicius

The optimum route for the synthesis of methyl esters of N-[(4-substituted amino)-5-cyano-2-methylthiopyrimidin-6-yl]amino acids (which are starting materials for preparing the methyl esters of the corresponding 5-amino-4-(substituted amino)pyrrolo[2,3-d]pyrimidine-6-carboxylic acids) is via subsequent reactions of 4,6-dichloro-2-methylthiopyrimidine-5-carbonitrile with amines and methyl glycinate. In some examples, the reaction of methyl N-(4-chloro-2-methylthio-6-pyrimidinyl)aminoacetate with amines occurs to give the corresponding acid amides. The previously unknown synthesized derivatives of pyrimidin-6-yl amino acids and 4,5-diaminopyrrolo[2,3-d]pyrimidine-6-carboxylic acids possess fungicidal properties.

Keywords: 4,5-diaminopyrrolo[2,3-*d*]pyrimidines, 4,6-dichloropyrimidine-5-carbonitrile, derivatives of N-4-substituted amino-5-cyanopyrimidin-6-ylaminoacetic acids, anticancer activity, fungicides.

Pyrrolo[2,3-*d*]pyrimidine derivatives, as 7-deaza analogs of biogenic purines, are of considerable interest in the scheme for the search for biologically active materials. This heterosystem is widely distributed in nature, being the part of antibiotics of tubercidin, toyocamycin, sangivamycin [1], cadeguomycin [2], rigidin alkaloids [3] etc. Many synthetic pyrrolo[2,3-*d*]pyrimidine derivatives show antiviral [4-8], anticancer [5, 6, 9-12], antioxidant, and neuroprotective activity [13], being inhibitors of dihydrofolic acid reductase [14]. Tricyclic condensed heterocycles which have a pyrrolo[2,3-*d*]pyrimidine fragment, also show biological activity [15-17].

We have recently reported the synthesis of esters of 4,5-diaminothieno[2,3-*d*]pyrimidine-6-carboxylic acids and their use in preparing the tricyclic, *peri*-annelated heterocycles polyazathiaacenaphthylenes [18-20]. In this work we have studied methods of synthesis of 7-aza analogs of the previously published thienopyrimidines in the case of the methyl esters of 4,5-diamino-2-methylthiopyrrolo[2,3-*d*]pyrimidine-6-carboxylic acids. One of the synthetic methods for esters of 4,5-diaminopyrrolo[2,3-*d*]pyrimidine-6-carboxylic acids is a Thorpe type cyclization reaction of the corresponding esters of N-[(4-substituted amino)-5-cyano-2-methylthiopyrimidin-6-yl]-aminoacetic acids. Synthesis of the latter from 4,6-dichloro-2-methylthiopyrimidine-5-carbonitrile (**1**) can be achieved via two routes which differ only in the sequence of the reaction of the pyrimidinecarbonitrile **1** with the aminoacetic ester and the amines.

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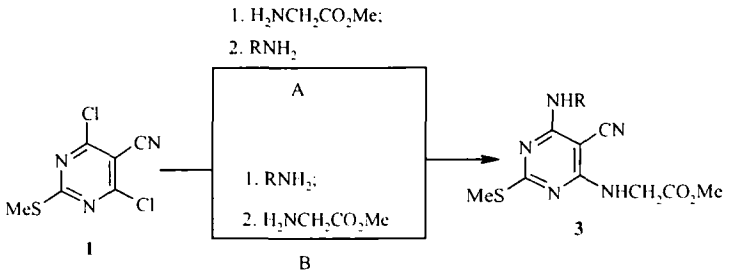
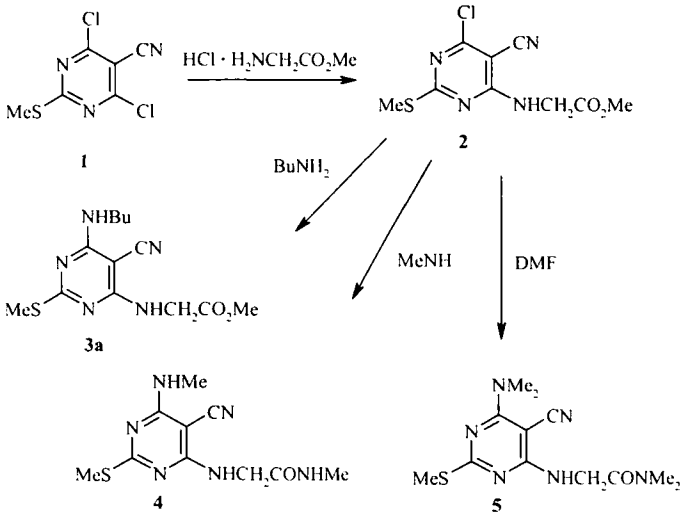


Data concerning the nucleophilic substitution of the chlorine atoms in the pyrimidine ring [21] and the synthesis of several N-(4-substituted 5-cyanopyrimidin-6-yl)aminoacetic acids [22] suggest that the introduction of a second amino group into the molecule calls for a more prolonged reaction time and more forcing conditions (due to the electron donor effect of the first) and that this usually lowers the yields of the target products. On the other hand, the initial introduction of an aminoacetic acid residue (having a less marked electron donor effect when compared with an amino group) can facilitate the exchange of the second chlorine atom by primary amines. For these reasons, it seemed to us worth comparing both synthetic routes for compound **3**.

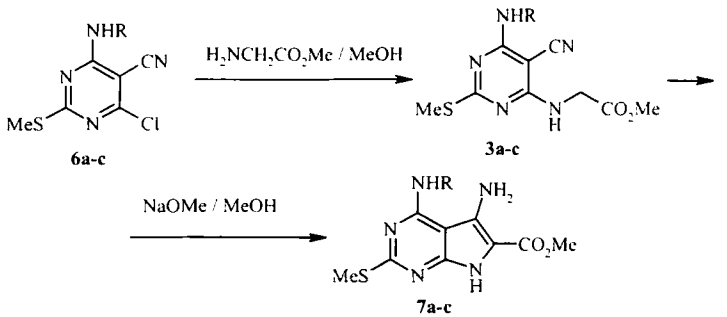


Treatment of the carbonitrile **1** with an excess of the methyl ether of aminoacetic acid at room temperature gave a compound which, according to IR and ^{1}H NMR data (Table 1), is the methyl ester of N-(4-chloro-5-cyano-2-methylthiopyrimidin-6-yl)aminoacetic acid (**2**). The reaction of compound **2** with butylamine, methylamine, and *o*-chloroaniline was also studied.

When heating the methyl ester **2** with a twofold molar excess of butylamine in methanol, the substitution of the chlorine in the 4 position takes 5 h and forms the corresponding 4-butylamino derivative **3a**. Compound **2** reacts more slowly with methylamine. After prolonged refluxing (12 h), a compound was produced whose IR spectrum showed a carbonyl group absorption band at 1672 cm^{-1} which was shifted to longer wavelength by 64 cm^{-1} when compared with the C=O group absorption in compound **2**. This points to the presence of an amido group in the molecule. The ^{1}H NMR spectrum of the compound obtained shows three broad singlets at 5.34, 5.88, and 6.15 ppm for the NH groups and two doublets at 2.83 and 3.05 ppm, corresponding to the methylamino groups at position 4 of the pyrimidine ring and in the amide group. Thus the spectroscopic data, along with the elemental analysis, show that the reaction of compound **2** with methylamine leads to the methylamide of N-(5-cyano-4-methylamino-2-methylthiopyrimidin-6-yl)aminoacetic acid. Introduction of an *o*-chlorophenylamino group into the pyrimidine ring by means of the reaction of compound **2** with *o*-chloroaniline was unsuccessful in our hands. The reaction does not occur upon prolonged heating in ethanol or decalin nor without solvent in a large excess of

o-chloroaniline at 190°C. When compound **2** is heated with *o*-chloroaniline in DMF, according to TLC data, there is formed a reaction product which can be separated in 27% yield after refluxing for 30 h. In the ^{1}H NMR spectrum of the compound obtained the aromatic proton signal is missing and, along with the signal for the CH_3 group, there are seen two singlets at 3.01 and 3.29 ppm of identical intensity amounting to six protons. The IR spectrum shows an amide type carbonyl group absorption at 1652 cm^{-1}. On the basis of this data we propose that the reaction product formed is the dimethylamide of N-(5-cyano-4-dimethylamino-2-methylthiopyrimidin-6-yl)aminoacetic acid (**5**). The structure of compound **5** was also confirmed by elemental analytical data. The occurrence of dimethylamine in the reaction mixture can be explained by a partial decomposition of DMF upon prolonged heating. Hence, the synthesis of esters of N-[(4-substituted amino)-5-cyano-2-methylthiopyrimidin-6-yl]amino acids **3** by method A is accompanied by side reactions in several cases. Thus we carried out a comparative study of the synthesis of the target compounds by method B via the intermediate 4-(substituted amino)-6-chloro-2-methylthiopyrimidine-5-carbonitriles **6** which had been obtained previously by reaction of carbonitrile **1** with the corresponding primary amines [18, 19].

It was found that refluxing compound **6** for 2.5-8 h with an excess amount of the methyl ester of aminoacetic acid in the presence of sodium carbonate gave the corresponding methyl esters **3a-c** in 60-82% yield. Upon heating with an equimolar amount of sodium methylate the compounds **3a-c** are converted to the pyrrolo[2,3-*d*]pyrimidine derivatives **7a-c**. The ^{1}H NMR spectra of compounds **7a-c** show a broad singlet for the NH_2 group in the region 4.69-5.95 ppm and the absence of a doublet signal for an NCH_2 group (as seen at 4.24-4.29 ppm in the spectra of compounds **3a-c**). The IR spectra of compounds **3a-c** show a CN group absorption band at 2200-2208 cm^{-1}. The absorption band for the ester group CO in the methyl esters **3a-c** is found at 1728-1752 cm^{-1} and in the IR spectra of the methyl esters of the pyrrolopyrimidines **7a-c** this band is shifted to longer wavelength and appears at 1664-1684 cm^{-1}.



a R = Bu, **b** Me, **c** *o*-ClC$_6$H$_4$

Hence the results obtained show that the more reliable and convenient method of synthesizing the methyl esters of 4,5-diamino-2-methylthiopyrrolo[2,3-*d*]pyrimidine-6-carboxylic acids from carbonitrile **1** is the stepwise reaction of the latter with amines and methyl glycinate and subsequent Thorpe cyclization of the obtained methyl N-(4-substituted amino-5-cyanopyrimidin-6-yl)aminoacetates in the presence of sodium methylate.

Several of the compounds synthesized in this work were investigated for their pesticidal and anticancer activity. Initial study of the pesticidal activity showed that compound **3b** showed fungicidal activity at a concentration of 500 ppm against *Botrytis cinerea* (80%), *Fusarium nivale* (60%), *Septoria nodorum* (55%), and *Pythium altinum* (50%). Compound **3c** shows activity at the same concentration against *Botrytis cinerea* (40%), *Fusarium nivale* (20%), *Pythium altinum* (30%), and *Colletotrichum gossypii* (30%). The activity of the pyrrolopyrimidine **7b** is towards *Fusarium nivale* (70%), *Septoria nodorum* (50%), and *Colletotrichum gossypii* (60%). The fungicidal activity of compound **7c** is somewhat higher than that of compound **3c**: *Fusarium nivale* (50%), *Pythium altinum* (40%), *Colletotrichum gossypii* (50%), and *Septoria nodorum* (45%). In addition, compound **7c** inhibits the growth of the screwworm by 50% at a concentration of 100 ppm.

TABLE 1. Characteristics of Compounds **2-5, 7, 8**

Compound	mp, °C (solvent)	IR spectrum, cm^{-1}	^1H NMR spectrum, δ, ppm*	Yield, %
2	166.5-168 (MeOH)	1736 (CO) 2216 (CN) 3280 (NH)	2.48 (3H, s, SCH_3); 3.79 (3H, s, OCH_3); 4.3 (2H, d, NCH_2); 6.2 (1H, t, NH)	92
3a	129-130.5 (2-PrOH)	1744 (CO) 2208 (CN) 3360 (NH)	0.92 (3H, t, CH_3); 1.44 (4H, m, CH_2CH_2); 2.42 (3H, s, SCH_3); 3.45 (2H, q, NCH_2); 3.76 (3H, s, OCH_3); 4.24 (2H, d, NCH_2); 5.29 (1H, t, NH); 5.71 (1H, t, NH)	76 (A) 82 (B)
3b	148-150 (MeOH)	1752 (CO) 2200 (CN) 3408 (NH)	2.44 (3H, s, SCH_3); 3.03 (3H, d, NCH_3); 3.76 (3H, s, OCH_3); 4.24 (2H, d, NCH_2); 5.28 (1H, br. s, NH); 5.67 (1H, br. t, NH)	60
3c	177.5-179 (dioxane–water)	1728 (CO) 2200 (CN) 3376 (NH)	2.45 (3H, s, SCH_3); 3.78 (3H, s, OCH_3); 4.29 (2H, d, NCH_2); 5.84 (1H, br. s, NH); 7.61 (4H, m, arom. protons, NH); 8.43 (1H, d, arom. protons)	71
4	196.5-198 ($CHCl_3$)	1672 (CO) 2220 (CN) 3336 (NH)	2.46 (3H, s, SCH_3); 2.83 (3H, d, NCH_3); 3.05 (3H, d, NCH_3); 4.12 (2H, d, NCH_2); 5.34 (1H, br. s, NH); 5.88 (1H, br. s, NH); 6.15 (1H, br. s, NH)	56
5	162-164 (MeOH)	1652 (CO) 2192 (CN) 3360 (NH)	2.45 (3H, s, SCH_3); 3.01 (6H, s, NCH_3); 3.29 (6H, s, NCH_3); 4.21 (2H, d, NCH_2); 6.62 (1H, br. t, NH)	27
7a	206-207 (MeOH)	1684 (CO) 3232, 3392 (NH, NH_2)	0.74 (3H, t, CH_3); 1.48 (4H, m, CH_2CH_2); 2.6 (3H, s, SCH_3); 3.45 (2H, q, NCH_2); 3.77 (3H, s, OCH_3); 4.95 (2H, s, NH_2); 6.47 (1H, s, NH); 7.23 (1H, s, NH)	68
7b	252-254 (dioxane–water)	1684 (CO) 3232, 3408 (NH, NH_2)	2.44 (3H, s, SCH_3); 2.90 (3H, s, NCH_3); 3.72 (3H, s, OCH_3); 5.95 (2H, s, NH_2); 7.21 (1H, br. s, NH); 10.45 (1H, s, NH)	53
7c	200-201 ($CDCl_3$)	1664 (CO) 3320, 3352, 3368 (NH, NH_2)	2.56 (3H, s, SCH_3); 3.89 (3H, s, OCH_3); 4.69 (2H, s, NH_2); 7.1 (3H, m, arom. protons); 8.01 (1H, s, NH); 8.4 (1H, s, NH); 8.82 (1H, d, arom. protons)	82

* ^1H NMR spectra were obtained for solutions in $CDCl_3$ (compounds **2-5, 7c**), C_5D_5N (compound **7a**), or DMSO-d_6 (compound **7b**).

TABLE 2. Elemental Analytical Data for Compounds Synthesized

Compound	Empirical formula	Found, % / Calculated, %		
		C	H	N
2	$C_9H_8ClN_4O_2S$	39.51 / 39.64	3.02 / 3.33	20.63 / 20.54
3a	$C_{13}H_{16}N_5O_2S$	50.64 / 50.47	6.33 / 6.19	22.49 / 22.64
3b	$C_{10}H_{13}N_5O_2S$	44.78 / 44.93	5.06 / 4.90	26.41 / 26.20
3c	$C_{15}H_{14}ClN_5O_2S$	49.75 / 49.52	3.98 / 3.88	18.97 / 19.25
4	$C_{10}H_{14}N_6OS$	45.25 / 45.10	5.23 / 5.30	31.72 / 31.56
5	$C_{12}H_{18}N_6OS$	49.11 / 48.96	6.02 / 6.16	28.45 / 28.55
7a	$C_{13}H_{16}N_5O_2S$	50.58 / 50.47	6.28 / 6.19	22.39 / 22.64
7b	$C_{10}H_{13}N_5O_2S$	44.69 / 44.93	4.71 / 4.90	26.32 / 26.20
7c	$C_{15}H_{14}ClN_5O_2S$	49.61 / 49.52	3.67 / 3.88	19.43 / 19.25

Testing of the anticancer activity of compounds **7b,c** *in vitro* were carried out at the National Cancer Institute (Bethesda, USA) and showed that they possess weak antitumor activity. The mean values of the inhibition of cell growth log GI_{50}, obtained after screening on 60 different types of malignant neoplasm cells, equal -4.07 and -4.72 respectively for compounds **7b** and **7c**. Compound **7c** is the most active (GI_{50} = 6.21 μM) relative to SK-MEL-5 melanoma cells.

EXPERIMENTAL

IR spectra were recorded in vaseline oil in a Specord M-80 instrument. ^{1}H NMR spectra were obtained on a Tesla BS-567 A spectrometer (80 MHz) using HMDS internal standard. Monitoring of the reaction course and the purity of the materials obtained was carried out by TLC on DC-Alufolien Aluminiumoxid 150 F254 neutral (type T) plates.

4,6-Dichloro-2-methylthiopyrimidine-5-carbonitrile (**1**) was prepared my method [23].

N-(4-Chloro-5-cyano-2-methylthiopyrimidin-6-yl)aminoacetic Acid Methyl Ester (2). A mixture of compound **1** (6 g, 27.3 mmol), the methyl ester of aminoacetic acid hydrochloride (6.83 g, 54.6 mmol), sodium carbonate (5.79 g, 54.6 mmol), and methanol (300 ml) was stirred at room temperature for 2.5 h. The precipitate was filtered off and recrystallized.

N-(4-Butylamino-5-cyano-2-methylthiopyrimidin-6-yl)aminoacetic Acid Methyl Ester (3a). A. A mixture of compound **2** (0.7 g, 2.57 mmol), butylamine (0.38 g, 5.2 mmol), and methanol (25 ml) was refluxed for 5 h. It was then cooled to room temperature and the precipitate was filtered off and recrystallized.

B. A mixture of the methyl ester of aminoacetic acid hydrochloride (3 g, 24 mmol), sodium carbonate (2.54 g, 24 mmol) and methanol (30 ml) was refluxed with stirring for 30 min. After cooling to room temperature, compound **6a** (2 g, 7.8 mmol) was added and refluxing continued for a further 7.5 h. The hot solution was then filtered from the inorganic salt and the filtrate was concentrated to one third initial volume, cooled, and the precipitate filtered off and recrystallized.

N-(5-Cyano-4-methylamino-2-methylthiopyrimidin-6-yl)aminoacetic Acid Methyl Ester (3b). Obtained similarly to the synthesis of compound **3a** by method B.

N-[4-(*o*-Chlorophenylamino)-5-cyano-2-methylthiopyrimidin-6-yl]aminoacetic Acid Methyl Ester (3c). A mixture of compound **6c** (1 g, 3.2 mmol), the methyl ester of aminoacetic acid hydrochloride (0.9 g, 7.2 mmol) sodium carbonate (0.76 g, 7.2 mmol), and methanol (50 ml) was refluxed for 2.5 h. After cooling to room temperature, the precipitate was filtered off and recrystallized.

N-(5-Cyano-4-methylamino-2-methylthiopyrimidin-6-yl)aminoacetic Acid Methylamide (4). A mixture of compound **2** (2 g, 7.35 mmol), aqueous methylamine (25%, 4 ml, 30 mmol), and methanol (50 ml) was refluxed for 12 h. After cooling to room temperature, the precipitate was filtered off and recrystallized.

N-(5-Cyano-4-dimethylamino-2-methylthiopyrimidin-6-yl)aminoacetic Acid Dimethylamide (5). A mixture of compound **2** (1 g, 3.68 mmol), *o*-chloroaniline (0.94 g, 7.4 mmol), and DMF (2 ml) was refluxed for 30 h. After cooling to room temperature, the precipitate was filtered off and recrystallized.

Methyl Esters of 5-Amino-4-(substituted amino)-2-methylthio-7H-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic Acids (7a-c). General method. The appropriate compound **3a-c** (1.43 mmol) was added to a solution of sodium methylate prepared from sodium (0.033 g, 1.43 mmol) and anhydrous methanol (25 ml). After refluxing for 3 h, the product was cooled to room temperature, and the precipitate was filtered off and recrystallized.

The authors express their sincere personal thanks to Uniroyal Chemical Ltd (Canada) for investigation of the pesticidal activity and the National Cancer Institute (Bethesda, USA) for carrying out the screening of the synthesized compounds for antitumor activity.

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