Synthesis of Some Polycyclic 1,2,4-Triazines Disposing of Acidic N-H Group [1]

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7-(6-Azauracil-5-yl)-isatin **1** was converted through its thiosemicarbazone **2** to 6-(6-azauracil-5-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indol-3-thione **3** and through the thiosemicarbazone of appropriate isatinic acid to 2-(2-thio-6-azauracil-5-yl)-6-(6-azauracil-5-yl)-aniline **4**. The course of the cyclocondensation of this compound was studied and the reaction was found to proceed in both possible ways, resulting in a mixture of compound **3** and regioisomer 6-(2-thio-6-azauracil-5-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]-indol-3-one **5**. Substituted aniline **4** was oxidized to 2,6-bis-(6-azauracil-5-yl)-aniline **7**, which served for the preparation of hydrazone **8**, cyclization of which led to 1-[2,6-bis-(6-azauracil-5-yl)-phenyl]-6-azauracil-5-carbonitrile **9**. This is the first tricyclic 6-azauracil with vicinal arrangement of 6-azauracil rings.

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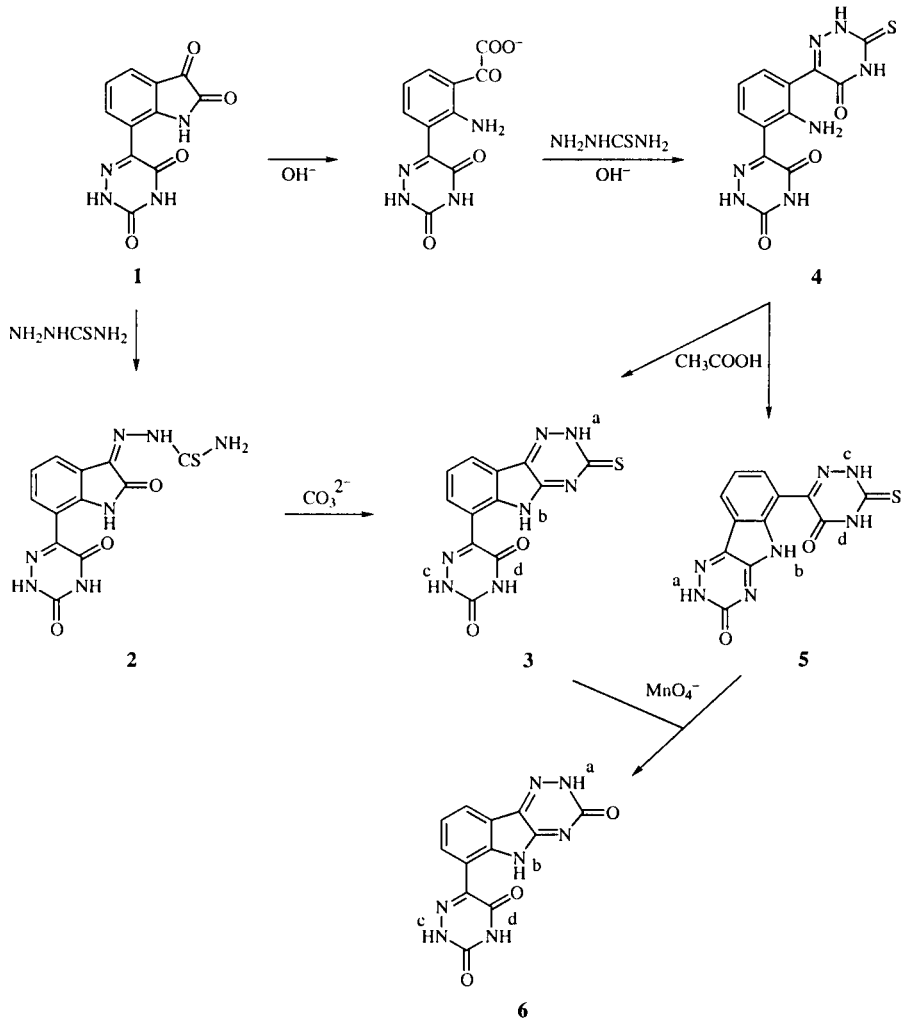
From the biological activity point of view, compounds which can interact with suitable substrates (e.g. proteins, nucleic acids) on the basis of intermolecular hydrogen bonds in two or more centers are interesting. Compounds

Scheme 1



of this type are very often able to block active centers of substrate molecules or can at least affect the conformation of these molecules. An important role among the molecules of this type is played by heterocycles containing N-H groups.

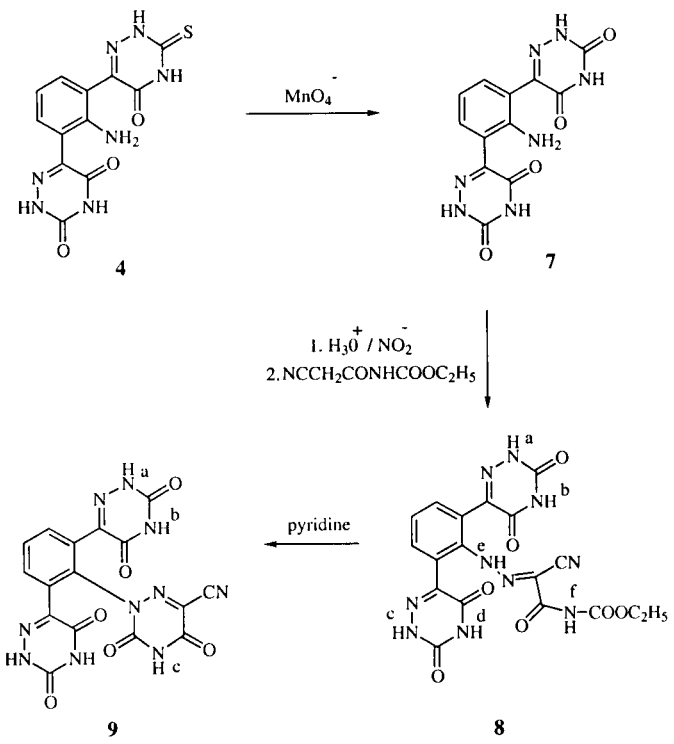
Very suitable model compounds, which could serve for study of intermolecular hydrogen bonds at two or more centers, are 1,2,4-triazines containing N-H groups. From this point of view polycyclic 6-azauracils can be very interesting. They can form intermolecular hydrogen bonds stronger than analogous uracils, because their acidity is higher by two orders of magnitude (pK of 6-azauracil is 7.00 [2,3], pK of uracil is 9.43 [3]).

We were engaged in the synthesis of some compounds of this type, reported in previous communications [4-6]. Earlier [4] we described a very convenient preparation of polycyclic 1,2,4-triazines, starting from 7-(6-azauracil-5-yl)-isatine. In continuation of this work, we now describe the synthesis of other compounds of this type.

Reaction of 7-(6-azauracil-5-yl)-isatine **1** [4] with thiosemicarbazide in a solution of sodium bicarbonate or dimethylformamide afforded in good yields the thiosemicarbazone **2** (Scheme 1). This compound is easily converted in refluxing alkaline solution to 6-(6-azauracil-5-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indole-3-thione **3**. The anion of the corresponding isatinic acid, prepared by alkaline opening of the substituted isatine **1** reacted with thiosemicarbazide to give 2-(6-azauracil-5-yl)-6-(2-thio-6-azauracil-5-yl)aniline **4**. The question of the regioselectivity of the cyclocondensation of disubstituted aniline **4** was very interesting. The question was whether the amino group would condense with the carbonyl group of the 6-azauracil ring or with the 2-thio-6-azauracil ring. Cyclocondensation was found to really proceed by both pathways. Therefore, a mixture of condensed isomeric derivatives **3** and 6-(2-thio-6-azauracil-5-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indol-3-one **5** was obtained. From NMR spectra the ratio of the isomers **3** to **5** was approximately 10:3. Permanganate oxidation converted this mixture to 6-(6-azauracil-5-yl)-2,3-dihydro-1,2,4-triazine-[5,6-*b*]indole-3-one, **6**.

The oxidation of thioderivative **4** leading to 2,6-bis-(6-azauracil-5-yl)-aniline **7** was also successful. We were interested in the extent of steric hindrance of the amino group placed among the 6-azauracil rings in this compound. Diazotization of compound **7** and subsequent coupling of the resulting diazonium salt with ethyl cyanoacetylcarbamate afforded hydrazone **8** in 63% yield. In refluxing pyridine this compound is converted to 1-[2,6-bis-(6-azauracil-5-yl)-phenyl]-6-azauracil-5-carbonitrile **9**, which is the first tricyclic 6-azauracil with vicinal arrangement of 6-azauracil rings (Scheme 2).

Scheme 2



Cyclization of compound **8** to compound **9** also occurs in the ion trap of the mass spectrometer. The full and zoom scans verified the molecular mass value (pseudomolecular ion 481 and 435 corresponding to the molecular mass 482 and 436 respectively), and the isotopic peak intensities correspond to the elemental composition of analyzed compounds. The collision dissociation of selected ions in the ion trap was performed. The ion 481 occurring in compound **8** affords the most intensive fragment 435 which corresponds to compound **9**. Its fragmentation is in agreement with fragmentation of compound **9**. Compounds **3-6** are composed of four different N-H groups, which can fill miscellaneous conformations, whereas compound **9** contains five N-H groups of three different types.

Compound **9** is now a subject of study of possible atropoisomery and interaction with modeling substrates.

EXPERIMENTAL

Melting points were determined on a Boetius stage and are not corrected. Infrared spectra were measured in potassium bromide disks and scanned on an ATI Unicam Genesis FTIR instrument. The nmr spectra were measured in solutions of DMSO-d_6 on a Bruker AMX-360 spectrometer (360 MHz) with TMS as an internal standard; the chemical shifts reported are in ppm. Elemental analyses were performed by using an EA 1108 Elemental Analyzer (Fison Instrument).

Mass spectra were obtained using an ion trap mass spectrometer LCQ Finnigan Mat equipped with electrospray ionization interface. Mass spectra were scanned in full scan regime, zoom scan and in MS^n experiments.

3-Thiocarbamoylhydrazono-7-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-2,3-dihydroindole-2-one (**2**).

Method I.

Substituted isatine **1** [4] (3 g; 11.61 mmol) was dissolved in refluxing solution of aqueous sodium bicarbonate (3 g; 28.3 mmol, 480 ml). After **1** dissolved, thiosemicarbazide (1.2 g; 13.29 mmol) was added and mixture was refluxed for 20 minutes. After cooling to the room temperature the solution was acidified with acetic acid and allowed to stand over night. The precipitate was filtered, washed with water and dried in air to yield 3.5 g (91%) of **2**.

Method II.

Substituted isatine **1** (0.8 g; 3.1 mmol) was dissolved in dimethylformamide (100 ml). Thiosemicarbazide (0.3 g; 3.3 mmol) was added to this solution and the mixture was heated at 80° for 5 hours. The solution was filtrated and diluted with water (300 ml). The resulting solid was filtered, washed with water and dried in air to yield 0.9 g (88%) of **2**. Recrystallization from ethanol and dimethylformamide (1:1 v/v) afforded the yellow solid, mp > 320°; ir: 3388, 3175 (NH), 1725, 1698, 1674 (C=O) cm^{-1}; ^1H nmr spectra: 7.21 (t, 1H, 5-H, J = 7.8 Hz); 7.52 (dd, 1H, 4-H, J = 7.8, 1.1 Hz); 7.79 (dd, 1H, 6-H, J = 7.8, 1.1 Hz); 8.79 (br s, 0.8H, NH_2); 9.14 (br s, 0.8H, NH_2); 11.06-12.9 (broad bands 3H, NH); ^{13}C nmr spectra: 116.52; 120.46; 121.65; 122.09; 131.49; 131.87; 140.56; 140.89; 149.44; 156.64; 162.53; 178.82.

Anal. Calcd. for $C_{12}H_9N_7O_3S$: C, 43.50; H, 2.74; N, 29.59. Found: C, 43.42; H, 2.64; N, 29.31.

6-(3,5-Dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indole-3-thione (**3**).

Compound **2** (0.6 g; 1.81 mmol) was refluxed in a solution of 5% sodium carbonate (60 ml) for 9 hours. After cooling to room temperature the solution was filtered and acidified with hydrochloric acid to *p*H 2. The resulting solid was filtered, washed with water and dried at 120° to yield 0.54 g (95%) of **3**. Recrystallization from acetic acid afforded the yellow solid, mp > 320°; ir: 3230 (NH), 3015 (ArH), 1690 (C=O), 1622 (NH) cm^{-1}; ^1H nmr spectra: 7.46 (t, 1H, 8-H, J = 7.7 Hz); 7.80 (dd, 1H, 9-H, J = 7.7, 1 Hz); 8.13 (dd, 1H, 7-H, J = 7.7, 1 Hz); 12.25 (2 x brs, 2H, a-, b-NH), 12.63 (s, 1H, c-NH), 14.68 (s, 1H, d-NH).

Anal. Calcd. for $C_{12}H_7N_7O_2S$: C, 46.00; H, 2.25; N, 31.30. Found: C, 45.72; H, 2.21; N, 30.72.

2-(3-Thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-6-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-aniline (**4**).

Substituted isatine **1** (1.3 g; 5.03 mmol) was dissolved in an aqueous solution of potassium hydroxide (1.3 g; 23.2 mmol, 40 ml). The solution was refluxed for 10 minutes, then thiosemicarbazide (0.46 g; 5.09 mmol) was added and refluxing continued for 15 minutes. After cooling to room temperature the mixture was filtered and acidified with acetic acid. The resulting solid was filtered and washed with water, then dissolved in aqueous potassium hydroxide (0.65 g; 11.5 mmol, 15 ml). A solution of 10% hydrochloric acid (20 ml) was added dropwise to this mixture. The solution was filtered and the filtrate neutralized with ammonium hydroxide to *p*H 7. The resulting solid was filtered, washed with water and dried in air to yield 1.2 g (70%) of **4**, mp > 320°; ir: 3348, 3205 (NH_2), 1706 (C=O) cm^{-1} ^1H nmr spectra: 5.60 (s, 2H, NH_2); 6.63 (t, 1H, 4-H, J = 7.6 Hz); 7.23 (dd, 1H, 5-H, J = 7.6, 1.6 Hz); 7.29 (dd, 1H, 3-H, J = 7.6, 1.6 Hz); 11.5-14.1 (broad bands, 4H, NH); ^{13}C nmr spectra: 114.16; 116.57; 117.53; 131.79; 132.27; 143.03; 145.69; 147.91; 149.73; 153.33; 157.16; 173.61.

Anal. Calcd. for $C_{12}H_9N_7O_3S$: C, 43.50; H, 2.74; N, 29.59. Found: C, 43.42; H, 2.64; 29.31.

6-(3,5-Dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-2,3-dihydro-5*H*-1,2,4-triazino[5,6-*b*]indole-3-one (**6**).

Method I.

Derivative **3** (354.6 mg, 1.13 mmol) was dissolved in aqueous potassium hydroxide (200 mg; 3.6 mmol, 20 ml). A solution of potassium permanganate (376.8 mg; 2.38 mmol) and sodium hydroxide (200 mg; 5 mmol) in water (20 ml) was added to this mixture. Mixture was stirred at room temperature for 30 minutes, then filtered. The precipitate of manganese dioxide was carefully washed with hot water and the combined filtrates were acidified with hydrochloric acid to *p*H 2 and refluxed for 5 minutes. The resulting solid was filtered, washed with water and dried. Yield: 309 mg (92%).

Method II.

Cyclization of 2-(3-Thioxo-5-oxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-6-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-aniline (**4**) to mixture of isomers **3** and **5.**

Compound **4** (330 mg; 1 mmol) was refluxed in acetic acid (10 ml) for 30 minutes. The solvent was evaporated to dryness to yield 303 mg (96.8%) of mixture **3** and **5** mp > 320°; ^1H nmr of compound **5**: 7.33 (t, 0.3H, 8-H, J = 7.5 Hz), 7.68 (d, 0.3H, 9-H, J = 7.5 Hz), 8.01 (d, 0.3H, 7-H, J = 7.5 Hz), 11.73 (br s, 0.3H, b-NH), 13.14 (br s, 0.2H, a-NH), 13.39 (br s, 0.2H, e-NH), 13.77 (br s, 0.2H, d-NH). ^1H nmr of compound **3**: 7.46 (t, 1H, 8-H, J = 7.7 Hz); 7.80 (dd, 1H, 9-H, J = 7.7, 1 Hz); 8.13 (dd, 1H, 7-H, J = 7.7, 1 Hz); 12.25 (2 x br s, 2H, a- and b-NH), 12.63 (s, 1H, c-NH), 14.68 (s, 1H, d-NH).

Oxidation of the Mixture of Isomeric Compounds **3** and **5** to Compound **6**.

Prepared mixture of **3** and **5** (250 mg; 0.8 mmol) was dissolved in aqueous potassium hydroxide (112 mg; 2 mmol, 10 ml). After dissolving a solution of potassium permanganate (269 mg; 1.7 mmol) and potassium hydroxide (121 mg; 2.2 mmol) in water (10 ml) was dropwise added. The mixture was stirred at room temperature for 30 minutes. Then it was filtered and the precipitate of manganese dioxide carefully washed with hot water. Combined filtrates were acidified with hydrochloric acid to *p*H 2 and refluxed for 5 minutes. The resulting solid was filtered, washed with water and dried in air to yield 211 mg (88.8%) of **6**, mp > 320°; ir: 3336, 3181 (NH), 1724, 1697 (C=O) cm^{-1}; ^1H nmr spectra 7.37 (t, 1H, 8-H, J = 7.7 Hz); 7.68 (dd, 1H, 9-H, J = 7.7, 1 Hz); 8.01 (dd, 1H, 7-H, J = 7.7, 1 Hz); 11.79 (s, 1H, b-NH); 12.25 (s, 1H, a-NH), 12.62 (s, 1H, c-NH); 13.17 (s, 1H, d-NH); ^{13}C nmr spectra: 117.92; 118.72; 121.94; 131.49; 133.56; 140.90; 141.80; 149.64; 155.07; 155.32; 156.87.

Anal. Calcd. for $C_{12}H_7N_7O_3$: C, 48.49; H, 2.37; N, 32.99. Found: C, 48.15; H, 2.11; N, 32.60.

2,6-Bis-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-aniline (**7**).

Compound **4** (900 mg; 2.7 mmol) was dissolved in aqueous potassium hydroxide (1 g; 17.8 mmol, 50 ml) with heating. After cooling a solution prepared from potassium permanganate (896 mg; 5.7 mmol), potassium hydroxide (1 g; 17.8 mmol) and water (20 ml) was added. The mixture was stirred for 30 minutes, then filtered, and the precipitate of manganese dioxide carefully washed with hot water. Combined filtrates were acidified with acetic acid to *p*H 4-5. The mixture was heated up to 70°, cooled and allowed to stand overnight. The precipitate was filtered, washed with water and dried in air to yield 720 mg (85%) of **7**. The properties of compound **7** are identical with the one prepared previously [4]. Ir: 3256, 3187 (NH_2), 3075 (ArH), 1737, 1714, 1691 (C=O) cm^{-1}, ^1H nmr spectra 5.45 (br s, 2H, NH_2); 6.63 (t, 1H, 4-H, J = 7.6 Hz); 7.22 (d, 2H, 3- and 5-H, J = 7.6 Hz), 11.7 - 12.6 (br s 2 x, 2.8H, NH).

Anal. Calcd. for $C_{12}H_9N_7O_4$: C, 45.72; H, 2.87; N, 31.10. Found: C, 45.83; H, 2.66; N, 30.73

Ethyl 2,6-Bis-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-phenylhydrazonocyanoacetylcarbamate (**8**).

Sodium nitrite (0.78 g; 11.3 mmol) was dissolved in 98% sulfuric acid (20 ml) at 0-5°. The mixture was stirred for 15 minutes and then heated up to 70°. After cooling to 0-5°, derivative **7** (2.06 g; 6.53 mmol) was slowly added and after dissolving the mixture was stirred another hour at this temperature. The mixture was then added dropwise to the solution of sodium hydroxide (44 g; 11 mmol) and ethyl cyanoacetylcarbamate (1.08 g; 6.9 mmol) in water (1:1) cooled to 0-5°. After addition was complete the *p*H was adjusted to the value 7-8 with acetic acid. After two weeks the mixture was acidified with hydrochloric acid to *p*H 2-3 and evaporated at room temperature to dryness. The residue was stirred with 300 ml of water and the solid was filtered, washed with water and dried in air to yield 1.99 g (63.2%) of **8**. Recrystallization from ethanol afforded the yellow solid, mp > 320°; ir: 3395, 3291 (NH), 2220 (CN), 1771, 1720, 1698 (C=O) cm^{-1}; ^1H nmr spectra: 1.28 (t, 3H, CH_3, J = 7.1 Hz); 4.16 (q, 2H, CH_2, J = 7.1 Hz); 7.51 (t,1H, 4-H, J = 7.6 Hz); 7.63 (d, 2H, 3- and 5-H, J = 7.6 Hz); 9.36 (s, 1H, e-NH); 11.92 (s,1H, f-NH); 12.17 (s, 2H, a-NH); 12.52 (s, 2H, b-NH); ^{13}C nmr spectra: 14.14; 61.63; 106.12; 110.23; 125.68; 125.83; 132.93; 138.38; 142.55; 149.35; 150.07; 156.82; 159.32; ms (m/z) (rel. int.): 481 (100), 435 (17), ms/ms 481 (m/z) (rel. int.): 435 (100), ms/ms/ms 435 (m/z) (rel. int.): 349 (17), 348 (16), 340 (14), 336 (24), 312 (12), 297 (66), 296 (100), 269 (14).

Anal. Calcd. for $C_{18}H_{14}N_{10}O_7$: C, 44.82; H, 2.93; N, 29.04. Found: C, 44.53; H, 2.86; N, 28.56.

2-[2,6-Bis-(3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-yl)-phenyl]-3,5-dioxo-2,3,4,5-tetrahydro-1,2,4-triazine-6-carbonitrile (**9**).

Derivative **8** (645 mg; 1.34 mmol) was refluxed in waterless pyridine (50 ml) for 12 hours. After that the solution was evaporated in a water bath to dryness. The residue was dissolved in 10% sodium carbonate (20 ml). The solution was filtered and acidified with hydrochloric acid to *p*H 2. The resulting solid was filtered, washed with water and dried in air to yield 430 mg (73.6%) of **9**. Recrystallization from a mixture of water and dimethylformamide (1:1 v/v) afforded the yellow solid, mp > 320°; ir: 3453 (NH), 2925, 2852 ($CH_{aliphatic}$), 2245 (CN), 1718, 1687 (C=O) cm^{-1}; ^1H nmr spectra: 7.71 (t, 1H, 4-H, J = 7.5 Hz); 7.79 (d, 2H, 3- and 5-H, J = 7.5 Hz); 12.11 (s, 2H, a-NH); 12.45 (s, 2H, b-NH); 12.55 (s, 0.5H, c-NH); ^{13}C nmr spectra: 111.84; 121.95; 128.92; 130.26; 132.37; 135.16; 139.84; 146.21; 148.78; 153.74; 155.92; ms (m/z) (rel. int.): 871 (100), 435 (73), ms/ms 435 (m/z) (rel. int.): 349 (24), 348 (23), 340 (21), 336 (36), 312 (19), 297 (85), 296 (100), 269 (15).

Anal. Calcd. for $C_{16}H_8N_{10}O_6$: C, 44.05; H, 1.85; N, 32.10. Found: C, 44.12; H, 1.92; N, 31.65.

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