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# Application of Fluorescent Triazoles to Analytical Chemistry. I. Determination of Aromatic Primary Amine with 2,4,6-Triaminopyrimidine as a Reagent

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A sensitive fluorometric method for the determination of primary aromatic amines was developed. The procedure requires diazotization of the amino group followed by coupling with 2,4,6-triaminopyrimidine (TAP), and the resulting azo compound is oxidized to a fluorescent triazole. Substituted triazoles were synthesized by using various pyrimidines and their fluorescence characteristics were evaluated. TAP was selected as the most sensitive reagent. None of the pyrimidines substituted at the 2-position was fluorescent. A method for the determination of sulfamethoxazole (SMX) was established (quantitation limit: 40 ng/ml). This method is more practical and simple than that reported before: the final solution for measurement was obtained in one flask through a one-pot reaction.

The effects of substituents of the triazole ring on the fluorescence are discussed.

**Keywords**——sulfamethoxazole; 2,4,6-triaminopyrimidine; fluorometric determination; triazolopyrimidine; *N*-bromosuccinimide; fluorescence characteristics; substituent effect; diazotization; primary aromatic amine

Several methods are available for the determination of primary aromatic amines, based on the fluorescence of a derivatized triazole. Dombrowski and Pratt[1] have reported a sensitive fluorometric method by using 2,6-diaminopyridine (DAP) as a reagent. Aniline was diazotized and coupled with DAP. The resulting azo derivative was oxidized with $CuSO_4$ oxidant to give a triazole. The method had the disadvantage that, since the reagent DAP is strongly fluorescent, it interfered with the assay. We subsequently established an improved assay method by nitrosating excess DAP to non-fluorescent 3-nitroso-2,6-diaminopyridine.[2] Our method, although simpler than Dombroski's, required the procedures of nitrosation and also extraction of the fluorophore.

We have now found that 2,4,6-triaminopyrimidine (TAP) is not fluorescent and is

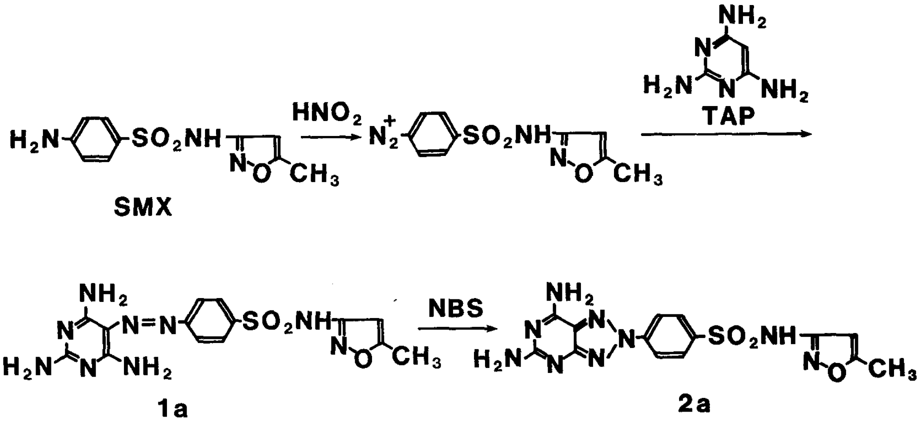


Chart 1

available for the determination of primary aromatic amines. The diazonium salt of sulfamethoxazole (SMX) was coupled with TAP, and the resulting azo derivative formed a triazole on oxidation. Not only $CuSO_4$ but also *N*-bromosuccinimide (NBS) as an oxidant afforded a triazole (**2a**), which was strongly fluorescent. A sensitive fluorometry was therefore established without the need for procedures to eliminate the effects of excess reagent and fluorescence quenching by $CuSO_4$.

The non-fluorescent reagent, 4-methoxy-*m*-phenylenediamine, which was found by Taniguchi *et al.*[3] has been used for the determination of primary aromatic amines in a method based on the same principle. Our method is comparable to their method in terms of sensitivity and simplicity, and does not require use of the pollutant $CuSO_4$.

This paper describes the assay method for SMX and the fluorescence characteristics of other triazoles derived from various 4,6-diaminopyrimidines substituted at the 2-position.

## Results and Discussion

### Conditions for the Determination of SMX

The excitation and emission spectra of the fluorophore **2a** are shown in Fig. 1. The spectra obtained by the assay procedure were in accord with those of **2a**.

### Diazo Coupling of SMX with TAP

**Effect of Temperature and Time on the Diazo Coupling**——The diazonium salt of SMX was coupled with TAP under various conditions, followed by oxidation. The intensity of the resulting fluorophore was measured. The rate of coupling was low at room temperature: 50%

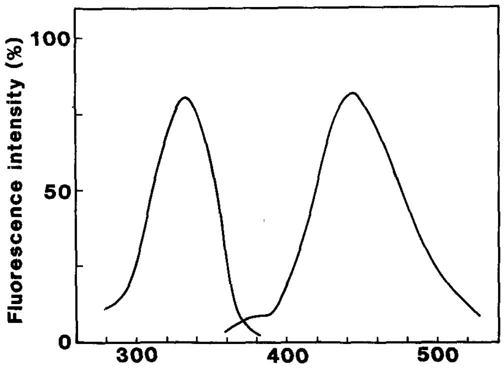


Fig. 1. Excitation and Emission Spectra of the Measurement Solution for SMX

A portion (1 ml) of the aqueous solution of SMX (0.5 μg/ml) was treated according to the assay procedure.

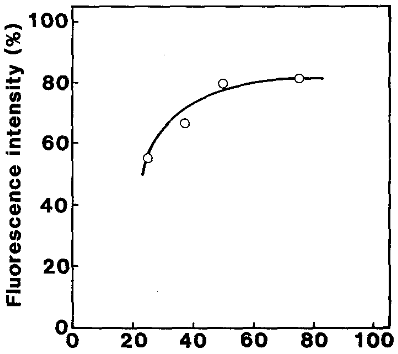


Fig. 2. Effect of Temperature on the Diazo Coupling

Portions (1 ml) of the aqueous solution of SMX (0.5 μg/ml) were treated according to the assay procedure but at various temperatures.

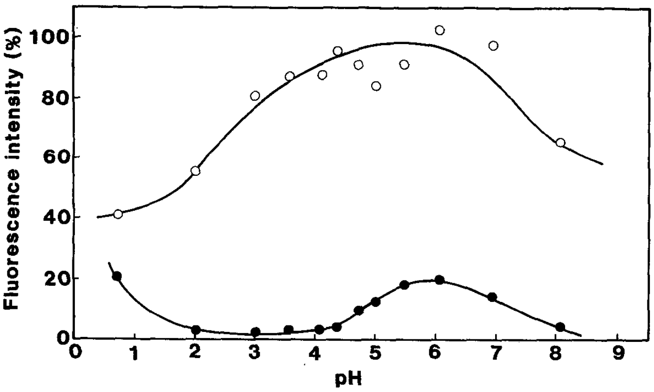


Fig. 3. Effect of pH on the Diazo Coupling

Portions (1 ml) of the aqueous solution of SMX (0.5 μg/ml) were treated according to the assay procedure but at various pH values.

○, triazole; ●, blank.

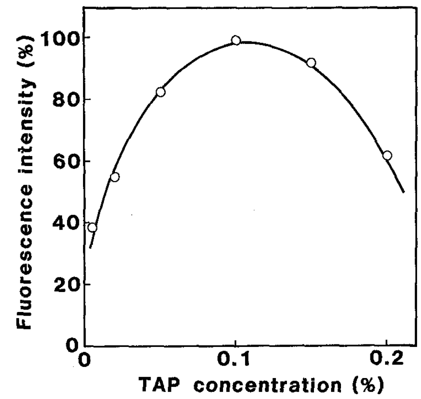


Fig. 4. Effect of TAP Concentration on the Diazo Coupling

Portions (1 ml) of the aqueous solution of SMX (0.5 $\mu$g/ml) were treated according to the assay procedure but with various concentrations of TAP.

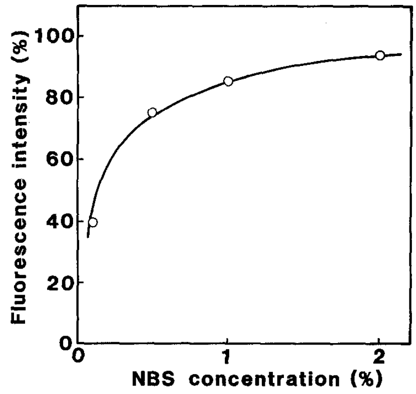


Fig. 5. Effect of NBS Concentration on the Oxidation

Portions (6 ml) of the aqueous solution of **1a** (0.125 $\mu$g/ml) were treated according to the assay procedure but with various concentrations of NBS.

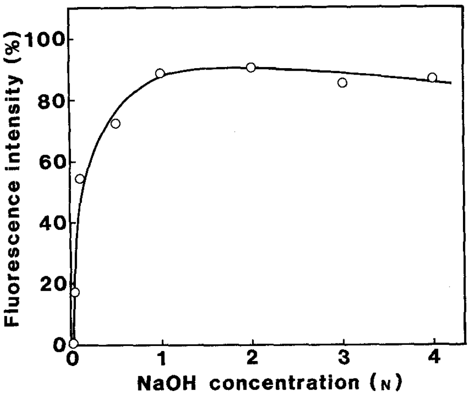


Fig. 6. Effect of NaOH Concentration on the Oxidation

Portions (6 ml) of the aqueous solution of **1a** (0.125 $\mu$g/ml) were treated according to the assay procedure but with various concentrations of NaOH.

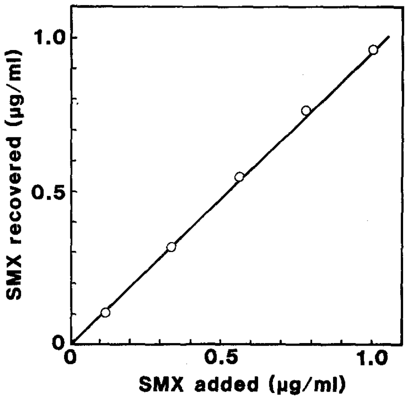


Fig. 7. Overall Recovery of SMX throughout the Assay Procedure

Poritions (1 ml) of the aqueous solution of SMX (0.04—1.0 $\mu$g/ml) were treated according to the assay procedure. Regression equation: $y=0.9681x-0.0064$, $s=0.0089$. The intercept was regarded as zero.

of SMX coupled with TAP in 30 min at 25 °C. The effect of temperature is shown in Fig. 2. The coupling yield increased with increase of the temperature, being 80% at 50—80 °C. The optimum reaction time was tested (15—60 min) at 50 °C using 0.1% TAP solution. A constant fluorescence intensity was observed over 30 min.

**Effect of pH on the Diazo Coupling**——The effect of pH was tested in the range of 1—8. As shown in Fig. 3, maximum fluorescence intensity was obtained at pH 4.4. A weak fluorescence due to the reagent TAP appeared at pH 6.

**Effect of Concentration of TAP**——Maximum intensity was obtained by the use of 0.1% solution (Fig. 4); the fluorescence decreased at higher concentrations.

## Oxidation of 1a

The triazole derivatives have generally been synthesized by the use of $CuSO_4$. This oxidant could not be applied in analysis because of its fluorescence quenching behavior. We found that NBS afforded **2a** as well as $CuSO_4$ did under mild conditions. This method consequently requires no separation procedure for $CuSO_4$ before fluorescence measurement.

In general, NBS is used in acidic solution as an oixdant. It was clarified that hypobromite

Table I. Fluorescence Characteristics of Sulfa Drug Solution under the Assay Conditions

| Sulfa drugs | Ex. (nm) | Em. (nm) | RFI (%) |
|---|---|---|---|
| Sulfamethoxazole | 333 | 440 | 100.0 |
| Sulfanilamide | 332 | 442 | 132.0 |
| Sulfaguanidine | 332 | 440 | 106.3 |
| Sulfisoxazole | 330 | 435 | 76.3 |
| Sulfathiazole | 335 | 443 | 40.4 |
| Sulfadimethoxine | 333 | 425 | 34.5 |
| Sulfisomidine | 337 | 437 | 10.1 |
| Sulfameradine | 335 | 420 | 10.1 |
| Sulfadiazine | 334 | 420 | 6.3 |
| Sulfaphenazole | 334 | 442 | 3.0 |

ion formed by the degradation of NBS in the alkaline solution oxidized **1a**. Compound **1a** was thus oxidized using sodium hypobromite solution instead of NBS solution. The same fluorophore was produced in almost the same yield.

**Effect of Concentrations of NBS and NaOH on the Oxidation**——Reagent NBS was dissolved in aqueous NaOH. The concentrations of both NBS and NaOH affected the oxidation yield. As shown in Fig. 5, a constant intensity was obtained by the use of 2% NBS solution. Figure 6 shows that the optimum concentration of NaOH was 1 N or above.

**Effect of Temperature and Time on the Oxidation**——Maximum and constant intensities were observed after more than 15 min and at 40 °C or above.

### Evaluation of the Assay Method

The overall recovery of SMX through the assay was estimated. Figure 7 shows that about 97% of SMX was recovered as **2a**. The amount of **2a** formed was determined by using a calibration curve obtained with standard solutions of **2a**.

Two amounts of SMX, 0.211 and 0.587 $\mu$g, were determined with coefficients of variation, 6.64% and 2.92% ($n = 10$/each), respectively. The detection limit was 40 ng/ml.

### Determination of Other Sulfa Drugs

Other sulfa drugs were subjected to the same assay procedure. All the drugs showed fluorescence similar to that of **2a**. The fluorescence characteristics are shown in Table I. The results show that sulfanilamide and sulfaguanidine, which fluoresced as strongly as SMX, can be determined by this method.

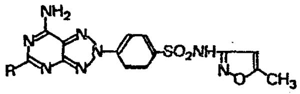
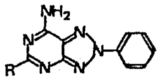
### Fluorescence Characteristics of the Triazole Derivatives

Some pyrimidotriazoles (**2a**—**f** and **4a**—**f**) having substituents at the 5-position were synthesized in order to find an analytical reagent with high sensitivity. Table II shows the fluorescence characteristics measured in two solvents. Of the compounds, **2a**—**f**, the 5-$NH_2$ derivative (**2a**) showed the most intense fluorescence in $H_2O$. The triazoles (**4a**—**f**) which were produced from aniline were also fluorescent. The fluorescence intensity of **4a** was twice that of **2a**. TAP may also be applicable to the determination of general primary aromatic amines.

The fluorescence of **2a**—**f** was greatly affected by pH. Since the compounds have a strongly acidic sulfamoyl group, dissociation in the ground and/or excited state of the group may have decreased the fluorescence. The compounds (**4a**—**f**) without a sulfamoyl group had a constant fluorescence intensity in neutral and alkaline solutions.

The fluorescence of the triazoles was also measured in dimethyl sulfoxide (DMSO). Compounds **2d** and **4d**, although they fluoresced weakly in $H_2O$, showed fluorescence as strong as that of **2a** and **4a**. In DMSO, it appeared that the stronger the electron donat-

Table II. Fluorescence Characteristics of Triazole Derivatives

| Solvent |  |  | Water[a) |  |  |  | DMSO |  |  |
|---|---|---|---|---|---|---|---|---|---|
| Triazole derivatives | R | Compd. No. | Ex. (nm) | Em. (nm) | RFI (%) | pH | Ex. (nm) | Em. (nm) | RFI (%) |
|  | $NH_2$ | **2a** | 333 | 440 | 100.0 | 2.7 | 380 | 461 | 73.0 |
|  | H | **2b** | 330 | 412 | 73.0 | 3.8 | 350 | 418 | 18.3 |
|  | $CH_3$ | **2c** | 333 | 419 | 43.2 | 4.2 | 351 | 420 | 1.5 |
|  | $N(CH_3)_2$ | **2d** | 380 | 479 | 8.8 | 8.0 | 395 | 478 | 67.0 |
|  | $SCH_3$ | **2e** | 350 | 435 | 9.4 | 2.7 | 361 | 426 | 1.0 |
|  | $C_6H_5$ | **2f** | 348 | 415 | —[b) | 3.6 | 361 | 422 | 4.7 |
|  | $NH_2$ | **4a** | 343 | 427 | 189.5 | 7.0 | 360 | 434 | 58.0 |
|  | H | **4b** | 320 | 392 | 89.0 | 7.0 | 330 | 391 | 2.3 |
|  | $CH_3$ | **4c** | 325 | 395 | 60.1 | 7.0 | 334 | 396 | 2.5 |
|  | $N(CH_3)_2$ | **4d** | 360 | 469 | 8.0 | 7.0 | 375 | 454 | 88.0 |
|  | $SCH_3$ | **4e** | 340 | 428 | 9.7 | 7.0 | 348 | 403 | 6.2 |
|  | $C_6H_5$ | **4f** | 335 | 399 | 12.6 | 7.0 | 350 | 398 | 15.9 |

*a*) Contained 1% DMSO. *b*) Unstable.

ing power of the substituent was, the more intense fluorescence the compounds showed. Introducing substituents on the 2-phenyl ring seemed to have little effect on the fluorescence. A search for more sensitive substituted pyrimidotriazole reagents is in progress.

## Experimental

**Apparatus**——A Hitachi MPF-2A recording spectrofluorometer was used for fluorescence measurement. A Taiyo M-IN incubator equipped with a shaker was used for the diazo coupling and the oxidation.

**Reagents and Materials**——Commercially available sulfa drugs, 2,4,6-triaminopyrimidine and 4,6-diaminopyrimidine were used. The other pyrimidines were prepared by the known method.[4) DMSO was purchased from Dojindo Laboratories (Luminasol®). The other chemicals were of reagent grade.

**Reagent Solution**——0.2% $NaNO_2$ Solution: Dissolve 0.2 g of $NaNO_2$ in $H_2O$ to make 100 ml.

1% Ammonium Sulfamate Solution: Dissolve 1 g of ammonium sulfamate in $H_2O$ to make 100 ml.

0.1% TAP Solution: Dissolve 0.02 g of TAP in $H_2O$ to make 20 ml.

2% NBS Solution: Dissolve 0.4 g of NBS in 2 N NaOH to make 20 ml and allow to stand for 1 h before use.

**Assay Procedure**——Weigh accurately about 5 mg of SMX into a 100-ml volumetric flask, dissolve it in 5 ml of 0.1 N NaOH and dilute to the mark with $H_2O$. Pipet 1 ml of the solution into a 100-ml volumetric flask and dilute to the mark with $H_2O$ (sample solution). Pipet 1 ml of the sample solution into a 10-ml volumetric flask. Add 0.5 ml of 2 N HCl and cool the flask in an ice-cold water. Add 0.5 ml of 0.2% $NaNO_2$ solution. Allow to stand for 15 min. Add 0.5 ml of 1% ammonium sulfamate solution and allow to stand for 15 min. Then, add 1.5 ml of pH 4.4 buffer solution (Britton–Robinson), 0.5 ml of 2 N NaOH and 0.5 ml of 0.1% TAP solution. Warm the flask in a water bath equipped with a shaker at 50 °C. Shake gently for 60 min. Add 1 ml of 2% NBS solution. Shake in a water bath at 50 °C for 25 min. After cooling to room temperature, add 1 ml of 2 N HCl and dilute with pH 3.5 buffer solution to make exactly 10 ml. After allowing to stand for 30 min, measure the fluorescence intensity at 440 nm with excitation at 333 nm, as well as that of the reagent blank prepared in the same manner using 1 ml of $H_2O$ instead of the sample solution.

**Calibration Curve**——A calibration curve was made according to the standard assay procedure. A linear relationship between the concentration of SMX and the fluorescence intensity was obtained in the range of 0.04—1.0 $\mu$g/ml.

**Examination of Conditions for the Oxidation Reaction**——An aliquot (1 ml) of the aqueous solution of **1a** (0.75 $\mu$g/ml) was placed in a 10-ml volumetric flask and 5 ml of $H_2O$ was added. This solution was tested under various reaction conditions (reagent concentration, time and temperature).

**4-[(2-Substituted 4,6-diaminopyrimidin-5-yl)azo]-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide**——An aqueous solution (10 ml) of $NaNO_2$ (2.5 g) was added dropwise to a solution of SMX (2.53 g; 0.01 mol) in 100 ml of 10% HCl under ice cooling. After 15 min, ammonium sulfamate (5 g) was added, and the mixture was stirred for 15 min. After addition of the pyrimidine (0.01 mol), the mixture was further stirred for 2 h. After neutralization with $NaHCO_3$, the precipitates were filtered off, washed with $H_2O$ and dried.

2-$NH_2$ (**1a**): Recrystallization from MeOH gave 2.32 g (57%) of **1a** as yellow prisms, mp 252—255 °C. IR (Nujol): 3515, 3460, 3415, 1658 $cm^{-1}$. *Anal.* Calcd for $C_{14}H_{15}N_9O_3S \cdot H_2O$: C, 41.27; H, 4.21; N, 30.94; S, 7.87. Found: C, 41.10; H, 4.35; N, 30.63; S, 7.77.

2-H (**1b**): Recrystallization from MeOH gave 1.19 g (31%) of **1b** as orange prisms, mp 278—280 °C. IR (Nujol): 3455, 3280, 1663, 1630 $cm^{-1}$. *Anal.* Calcd for $C_{14}H_{14}N_8O_3S \cdot 1/2H_2O$: C, 43.86; H, 3.94; N, 29.23; S, 8.36. Found: C, 43.90; H, 4.03; N, 29.33; S, 8.60.

2-$CH_3$ (**1c**): Recrystallization from MeOH gave 2.83 g (76%) of **1c** as yellow prisms, mp 290—292 °C. IR (Nujol): 3470, 3280, 1610 $cm^{-1}$. *Anal.* Calcd for $C_{15}H_{16}N_8O_3S$: C, 46.39; H, 4.15; N, 28.85; S, 8.26. Found: C, 46.31; H, 4.22; N, 28.65; S, 8.42.

2-$NMe_2$ (**1d**): Recrystallization from MeOH gave 2.76 g (66%) of **1d** as orange prisms, mp 278—280 °C. IR (Nujol): 3475, 3320, 1610 $cm^{-1}$. *Anal.* Calcd for $C_{16}H_{19}N_9O_3S$: C, 46.03; H, 4.59; N, 30.20; S, 7.68. Found: C, 46.19; H, 4.89; N, 30.04; S, 7.93.

2-SMe (**1e**): Recrystallization from MeOH gave 1.98 g (47%) of **1e** as orange prisms, mp 282—284 °C. IR (Nujol): 3480, 3300, 1620 (br) $cm^{-1}$. *Anal.* Calcd for $C_{15}H_{16}N_8O_3S_2$: C, 42.84; H, 3.84; N, 26.65; S, 15.25. Found: C, 42.72; H, 3.91; N, 26.78; S, 15.00.

2-Ph (**1f**): Recrystallization from MeOH gave 2.97 g (66%) of **1f** as red prisms, mp over 300 °C. IR (Nujol): 3475, 3360, 1615 $cm^{-1}$. *Anal.* Calcd for $C_{20}H_{18}N_8O_3S$: C, 53.33; H, 4.03; N, 24.87; S, 7.12. Found: C, 53.27; H, 3.83; N, 24.59; S, 7.25.

**4-(5-Substituted 7-amino-*v*-triazolo[4,5-*d*]pyrimidin-2-yl)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide**——The compounds (**2a**—**f**) were prepared by oxidation of 100 mg of the azo compounds (**1a**—**f**) by the method of Hartzel and Benson.[5)]

5-$NH_2$ (**2a**): Recrystallization from MeOH gave 59 mg (60%) of **2a** as colorless prisms, mp over 300 °C. IR (Nujol): 3600, 3100 (br), 1650 (br) $cm^{-1}$. *Anal.* Calcd for $C_{14}H_{13}N_8O_3S$: C, 43.41; H, 3.38; N, 32.54; S, 8.28. Found: C, 43.22; H, 3.44; N, 32.46; S, 8.16.

5-H (**2b**): Recrystallization from MeOH gave 38 mg (40%) of **2b** as colorless prisms, mp 277—280 °C. IR (Nujol): 3300, 1620 $cm^{-1}$. *Anal.* Calcd for $C_{14}H_{12}N_8O_3S$: C, 45.16; H, 3.25; N, 30.09; S, 8.61. Found: C, 44.88; H, 3.39; N, 29.76; S, 8.80.

5-$CH_3$ (**2c**): Recrystallization from MeOH gave 29 mg (30%) of **2c** colorless needles, mp over 300 °C. IR (Nujol): 3440, 3230, 1663 $cm^{-1}$. *Anal.* Calcd for $C_{15}H_{14}N_8O_3S$: C, 46.63; H, 3.65; N, 29.00; S, 8.30. Found: C, 46.40; H, 3.61; N, 28.81; S, 8.39.

5-$NMe_2$ (**2d**): Recrystallization from MeOH gave 19 mg (20%) of **2d** as pale yellow needles, mp over 300 °C. IR (Nujol): 3440, 3180 (br), 1640 (br) $cm^{-1}$. *Anal.* Calcd for $C_{16}H_{17}N_9O_3S$: C, 46.26; H, 4.12; N, 30.34; S, 7.72. Found: C, 46.52; H, 3.96; N, 30.24; S, 7.71.

5-SMe (**2e**): Recrystallization from MeOH gave 39 mg (40%) of **2e** as colorless prisms, mp 276—278 °C. IR (Nujol): 3100 (br), 1668, 1570 $cm^{-1}$. *Anal.* Calcd for $C_{15}H_{14}N_8O_3S_2$: C, 43.05; H, 3.37; N, 26.78; S, 15.32. Found: C, 43.09; H, 3.37; N, 26.49; S, 15.23.

5-Ph (**2f**): Recrystallization from MeOH gave 29 mg (30%) of **2f** as colorless prisms, mp 280—282 °C (dec.). IR (Nujol): 3440, 3200 (br), 1633 $cm^{-1}$. *Anal.* Calcd for $C_{20}H_{16}N_8O_3S$: C, 53.56; H, 3.60; N, 24.99; S, 7.15. Found: C, 53.50; H, 3.49; N, 24.77; S, 7.21.

**2-Substituted 4,6-Diamino-5-phenylazopyrimidine**——The compounds were prepared by diazo coupling of 2-substituted 4,6-diaminopyrimidines with aniline diazonium salt (method A) or by condensation of substituted amidine hydrochlorides with phenylmalononitrile (method B).

Method A: An aqueous solution (15 ml) of $NaNO_2$ (1 g) was added dropwise to a solution of aniline (0.93 g; 0.01 mol) in 30 ml of HCl (*ca.* 35%) under ice cooling. After 15 min, ammonium sulfamate (2 g) was added and the mixture was stirred for 15 min. After addition of the pyrimidine (0.01 mol), the mixture was further stirred for 2 h and then neutralized with $NaHCO_3$. The precipitates were filtered off, washed with $H_2O$, and dried.

2-$NH_2$ (**3a**): Recrystallization from DMF–$H_2O$ gave 1.98 g (80%) of **3a** as yellow prisms, mp 265—270 °C (dec.). IR (Nujol): 3320 (br), 1660 $cm^{-1}$. *Anal.* Calcd for $C_{10}H_{11}N_7 \cdot H_2O$: C, 48.58; H, 5.30; N, 39.65. Found: C, 48.32; H, 5.51; N, 39.30.

2-$NMe_2$ (**3d**): Recrystallization from DMF–$H_2O$ gave 1.74 g (68%) of **3d** as orange leaves, mp 146.5—147.5 °C. IR (Nujol): 3400, 1630 $cm^{-1}$. *Anal.* calcd for $C_{12}H_{15}N_7$: C, 56.02; H, 5.88; N, 38.11. Found: C, 55.99; H, 5.93; N, 37.70.

2-SMe (**3e**): Recrystallization from DMF–$H_2O$ gave 1.27 g (49%) of **3e** as brown prisms, mp 275—280 °C (dec.). IR (Nujol): 3480, 3300, 1620 (br) $cm^{-1}$. *Anal.* Calcd for $C_{11}H_{12}N_6S$: C, 50.75; H, 4.65; N, 32.28; S, 12.32. Found: C, 50.81; H, 4.54; N, 32.00; S, 12.40.

Method B: The substituted amidine hydrochloride (0.01 mol) and benzeneazomalononitrile (1.72 g, 0.01 mol) were dissolved in EtONa solution (0.25 g of sodium in 30 ml of EtOH). After standing for 1.5 h, the mixture was refluxed for 1.5 h, then allowed to stand overnight. The precipitates were filtered off, washed with $H_2O$ and dried.

2-H (**3b**): Recrystallization from pyridine gave 0.95 g (45%) of **3b** as yellow leaves, mp over 300 °C. IR (Nujol):

3470, 3320, 3280, 1630 cm$^{-1}$. *Anal.* Calcd for $C_{10}H_{10}N_6$: C, 56.07; H, 4.71; N, 39.23. Found: C, 56.25; H, 4.63; N, 39.26.

2-$CH_3$ (**3c**): Recrystallization from pyridine gave 0.66 g (29%) of **3c** as yellow leaves, mp over 300 °C. IR (Nujol): 3470, 3280, 1630, 1550 cm$^{-1}$. *Anal.* Calcd for $C_{11}H_{12}N_6$: C, 57.88; H, 5.30; N, 36.82. Found: C, 58.12; H, 5.09; N, 36.67.

2-Ph (**3f**): Recrystallization from pyridine gave 1.39 g (48%) of **3f** as yellow leaves, mp over 300 °C. IR (Nujol): 3435, 3280, 3080 (br), 1620 (br) cm$^{-1}$. *Anal.* Calcd for $C_{16}H_{14}N_6$: C, 66.19; H, 4.86; N, 28.95. Found: C, 66.47; H, 4.97; N, 28.82.

**5-Substituted 7-Amino-2-phenyl-*v*-triazolo[4,5-*d*]pyrimidine**——The compounds (**4a**—**f**) were prepared by oxidation of 100 mg of the azo compounds (**3a**—**f**) by the above method.[5]

5-$NH_2$ (**4a**): Recrystallization from DMF–$H_2O$ gave 28 mg (30%) of **4a** as colorless needles, mp over 300 °C. IR (Nujol): 3400, 3200, 1640 cm$^{-1}$. *Anal.* Calcd for $C_{10}H_9N_7$: C, 52.86; H, 3.99; N, 43.15. Found: C, 52.84; H, 4.09; N, 42.90.

5-H (**4b**): Recrystallization from DMSO–$H_2O$ gave 30 mg (32%) of **4b** as colorless needles, mp over 300 °C. IR (Nujol): 3040 (br), 1683, 1607 cm$^{-1}$. *Anal.* Calcd for $C_{10}H_8N_6$: C, 56.60; H, 3.80; N, 39.60. Found: C, 56.70; H, 3.74; N, 39.45.

5-$CH_3$ (**4c**): Recrystallization from MeOH gave 50 mg (52%) of **4c** as colorless needles, mp 252—253 °C. IR (Nujol): 3440, 3320, 1660 cm$^{-1}$. *Anal.* Calcd for $C_{11}H_{10}N_6$: C, 58.40; H, 4.46; N, 37.15. Found: C, 58.71; H, 4.53; N, 37.00.

5-$NMe_2$ (**4d**): Recrystallization from DMF–$H_2O$ gave 56 mg (58%) of **4d** as pale green needles, mp 270—271 °C. IR (Nujol): 3440, 3200, 1633 cm$^{-1}$. *Anal.* Calcd for $C_{12}H_{13}N_7$: C, 56.46; H, 5.13; N, 38.41. Found: C, 56.78; H, 5.21; N, 38.25.

5-SMe (**4e**): Recrystallization form DMSO–$H_2O$ gave 43 mg (45%) of **4e** as colorless needles, mp 273—274 °C. IR (Nujol): 3435, 3100, 1650 cm$^{-1}$. *Anal.* Calcd for $C_{11}H_{10}N_6S$: C, 51.15; H, 3.90; N, 32.54; S, 12.41. Found: C, 51.38; H, 3.96; N, 32.63; S, 12.48.

5-Ph (**4f**): Recrystallization from DMF–$H_2O$ gave 57 mg (58%) of **4f** as pale green leaves, mp 280—282 °C. IR (Nujol): 3440, 3320 (br), 1630 cm$^{-1}$. *Anal.* Calcd for $C_{16}H_{12}N_6$: C, 66.66; H, 4.20; N, 29.15. Found: C, 66.83; H, 4.36; N, 28.94.

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