Supplementary Information

An efficient and mild oxidant for the synthesis of s-tetrazines

Ramajeyam Selvaraj[a] , Joseph M. Fox[a]*

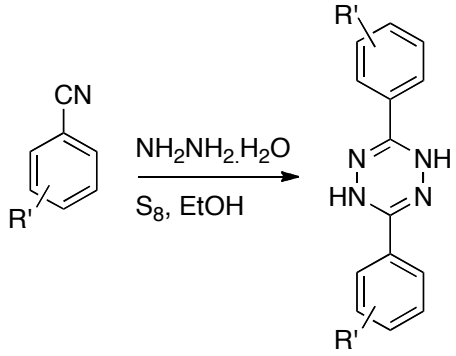
[a]*Department of Chemistry and Biochemistry, University of Delaware, Newark DE 19716*

E-mail: jmfox@udel.edu

Experimental section

General Considerations: All reactions were performed in round bottomed flasks or reaction vials. All commercially available reagents were used as received without further purification. Chromatography was performed on silica gel (Silicycle 40-63D, 60Å).

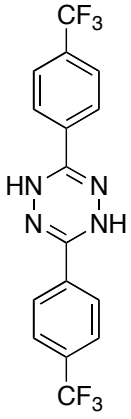
General procedure for dihydrotetrazine synthesis



This procedure was adapted from the literature.[1]

A round bottomed flask was equipped with a reflux condenser and a septum-fitted gas inlet adapter. Anhydrous ethanol (3 mL), the nitrile (10 mmol) and sulfur (200 mg) were added, and the apparatus was flushed with nitrogen atmosphere. Hydrazine monohydrate (2 mL) was added dropwise at 0 °C, and the resulting mixture was allowed to stir for 2 h while warming to r.t. The mixture was then refluxed for 2 h, cooled to 0 °C and filtered. The filtered solid was rinsed with cold ethanol (3 x 5 mL) to afford the dihydrotetrazine product, which was used in the subsequent oxidation step without further purification.

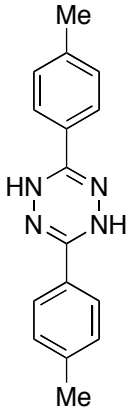
3,6-Bis(4-(Trifluoromethyl)phenyl)-1,4-dihydro-1,2,4,5-tetrazine (9e)



The general procedure for dihydrotetrazine synthesis with 4-trifluoromethylbenzonitrile gave 1.2 g (65%) of the title compound, a yellow solid, in 85% purity as judged by ^{1}H NMR analysis and the corrected yield was 55% (2.76 mmol). The material was used in the following step without further purification. Small impurity peaks could be observed by ^{1}H NMR at 8.28 and 7.96 ppm and by ^{13}C NMR at 167.4, 153.7, 129.1 and 126.6 ppm. mp: 214 °C (decomp); ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.40 (s, 2H), 8.03 (d, J = 8.3, 4H),

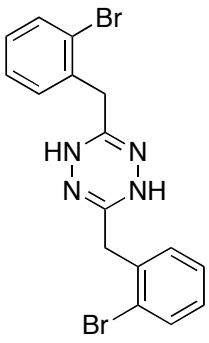
7.83 (d, J = 8.3, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 146.9 (u), 133.9 (u), 130.2 (q, ^{2}J(CF) = 32.0 Hz) (u), 126.8 (dn), 125.6 (q, ^{3}J(CF) = 3.4 Hz) (dn), 124.0 (q, ^{1}J(CF) = 272 Hz) (u); IR (neat, KBr, cm^{-1}): 3285, 2938, 1620, 1436, 1322, 1139, 1074; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd. for $C_{16}H_{10}F_6N_4^+$ 372.0805; found 372.0816.

3,6-Di-*p*-tolyl-1,4-dihydro-1,2,4,5-tetrazine (9f)



The general procedure for dihydrotetrazine synthesis with 4-cyanotoluene gave 864 mg of a yellow solid. The material was 83% pure, as judged by ^{1}H NMR analysis and the corrected yield was 54% (2.7 mmol). The material was used in the following step without further purification. Small impurity peaks could be observed by ^{1}H NMR at 7.91 and 7.40 ppm and by ^{13}C NMR at 167.9, 141.9, 130.5, 128.0 and 120.3 ppm. mp: 217 °C (decomp); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (s, 1H), 7.72 (d, J = 8.1 Hz, 2H), 7.26 (d, J = 8.1 Hz, 2H), 2.34 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.4 (u), 140.3 (u), 129.5 (dn), 127.9 (u), 126.3 (dn), 21.4 (dn); IR (neat, KBr, cm^{-1}): 3322, 2918, 1642,1418, 1335, 1286, 872, 726; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd for $C_{16}H_{16}N_4^+$ 264.1370; found 264.1371

3,6-Bis(2-bromobenzyl)-1,4-dihydro-1,2,4,5-tetrazine (9g)

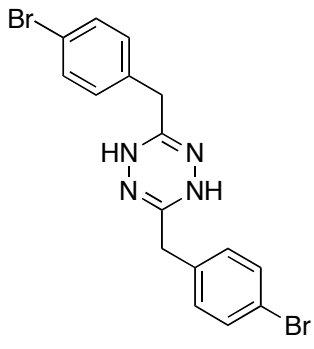


The general procedure for dihydrotetrazine synthesis with 2-(2-bromophenyl)acetonitrile) gave 430 mg (1.0 mmol, 20%) of the title compound as a white solid. The low yield was partially due to the solubility of the dihydrotetrazine in ethanol (higher yields could be obtained using the procedure for tetrazine synthesis without purification of the intermediate dihydrotetrazine, *vide infra*).

The dihydrotetrazine **9g** was 95% pure, as judged by ^{1}H NMR analysis.

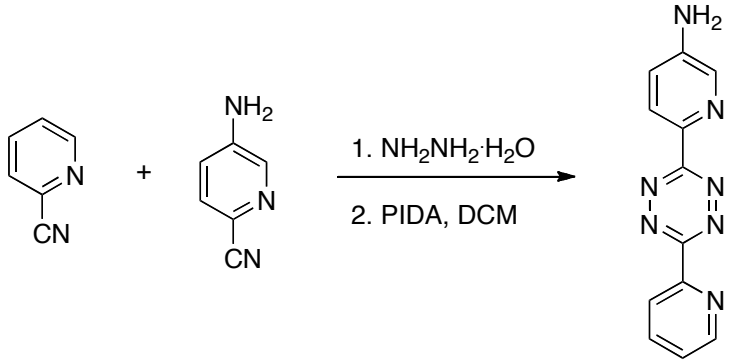
The material was used in the following step without further purification mp: 176 - 179 °C. Small impurity peaks could be observed by ^{13}C NMR at 128.1, 129.5 132.6 and 132.7 ppm. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10 (s, 2H), 7.59 (dd, *J* = 7.9, 1.0 Hz 2H), 7.41 (dd, *J* = 7.6, 1.6 Hz 2H), 7.33 (td, *J* = 7.6, 1.1 Hz 2H), 7.19 (td, *J* = 7.6, 1.6 Hz 2H), 3.47 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.2 (u), 135.8 (u), 132.3 (dn), 131.1 (dn), 128.7 (dn), 127.6 (dn), 124.4 (u), 36.3 (u); IR (neat, KBr, cm^{-1}): 3271, 3187, 3060, 2967 1568, 1439, 1234, 747, 600 ; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd for $C_{16}H_{14}Br_2N_4^+$ 421.9559; found 421.9565.

3,6-Bis(4-bromobenzyl)-1,4-dihydro-1,2,4,5-tetrazine (9h)



The general procedure for dihydrotetrazine synthesis with 2-(4-bromophenyl)acetonitrile) gave 780 mg (1.85 mmol, 37%) of the title compound as a white solid. There were material losses due to the solubility of the dihydrotetrazine in ethanol, and a higher yield could be obtained through the procedure for tetrazine synthesis without purification of the intermediate dihydrotetrazine, *vide infra*. The dihydrotetrazine **9h** was 98% pure, as judged by ^{1}H NMR analysis. The material was used in the following step without further purification mp: 211 °C (decomp). A small impurity peak could be observed by ^{13}C NMR at 131.9 and 132.1 ppm. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.02 (s, 2H), 7.50 (d, *J* = 8.4, 4H), 7.22 (d, *J* = 8.4, 4H), 3.32 (s, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 149.4 (u), 136.3 (u), 131.6 (dn), 131.5 (dn), 120.1 (u), 35.7 (u); IR (neat, KBr, cm^{-1}): 3353, 3276, 3150, 1663, 1487, 1412, 1324, 1233, 1207, 843, 584; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ Calcd for $C_{16}H_{14}Br_2N_4^+$ 421.9559; found 421.9562

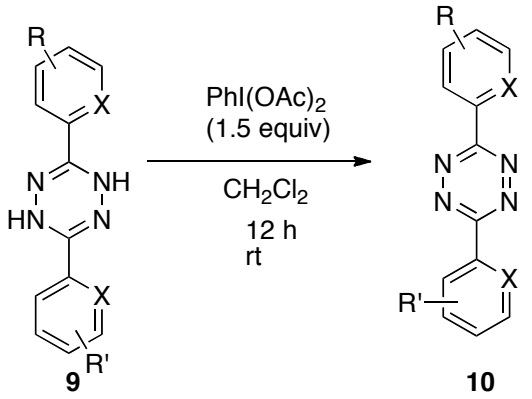
One pot synthesis of 6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine (2):



2

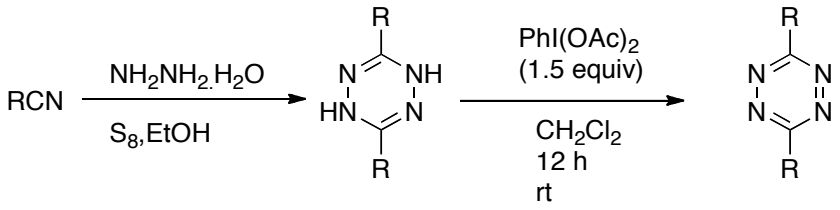
This procedure was carried out behind a blast shield. A 100 mL single neck round-bottomed flask was charged with 5-amino-2-cyanopyridine (0.57 g, 4.8 mmol). In a separate flask, 2-cyanopyridine (1.0 g, 0.93 mL, 9.6 mmol) was melted by gentle warming, and added to the flask containing 5-amino-2-cyanopyridine. Hydrazine hydrate (1.5 g, 1.5 mL, 31 mmol) was added, and the flask was fitted with reflux condenser and heated to 90 °C for 12 h under an atmosphere of nitrogen. The reaction mixture was cooled to r.t. and 20 mL of ice chilled water was added. A solid formed, which was broken into smaller pieces using a spatula, and then filtered on a Büchner funnel and rinsed with ice-cold water (20 mL). A spatula was used to break the solid into a powder, which suction dried on the Büchner funnel and then transferred to a round bottomed flask. Methylene chloride (50 mL) was added, and the suspended solid stirred. To the stirring suspension was added phenyliodonium diacetate (2.6 g, 8.1 mmol). The reaction mixture was allowed to stir for 2 h, and then adsorbed onto silica gel (9 g). Column chromatography using a gradient of 100% dichloromethane to 10% MeOH in dichloromethane as the eluent gave 6-(6-(pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)pyridin-3-amine **2** (0.53 g, 2.1 mmol, 44%) as a red solid (R_f = 0.52). Also formed was 3,6-(dipyridin-2yl)-1,2,4,5-tetrazine (R_f = 0.57). An identical experiment provided 0.51 g (2.0 mmol, 42%) of **2**. The spectral data for the title compound matches with the literature report.[2]

General procedure for the oxidation of dihydrotetrazines:



To a 20 mL reaction vial containing a stir bar was added phenyliodonium diacetate (1.5 mmol, 1.5 equiv) and dichloromethane (5 mL). To the stirring solution was added the appropriate dihydrotetrazine (1.0 mmol). The resulting mixture was allowed to stir at r.t. for 12 h and subsequently purified by column chromatography.

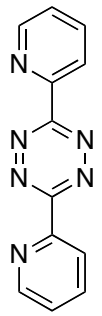
Procedure for tetrazine synthesis without purification of the intermediate dihydrotetrazine



A round bottomed flask was equipped with a reflux condenser and a septum-fitted gas inlet adapter. Anhydrous ethanol (1 mL), the nitrile (2 mmol) and sulfur (40 mg) were added, and the apparatus was flushed with nitrogen atmosphere. Hydrazine monohydrate (0.4 mL) was added dropwise at 0 °C, and the resulting mixture was allowed to stir for 2 h while warming to r.t. The mixture was then refluxed for 2 h, cooled to 0 °C diluted with water (10 mL) and extracted with dichloromethane (3 x 20 mL). The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and concentrated on rotary evaporator. The residue was dissolved in dichloromethane (10 mL) and transferred to a 20 mL reaction vial containing a stir bar.

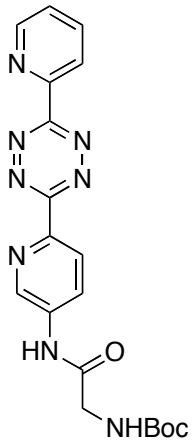
Phenyliodonium diacetate (1.5 mmol, 1.5 equiv) was added into the reaction vial, stirred at r.t. for 12 h and subsequently purified by column chromatography.

3,6-(Dipyridin-2-yl)-1,2,4,5-tetrazine (10a):



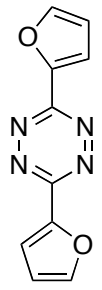
The general procedure for oxidation was followed using 3,6-di(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazine[3] (238 mg, 1.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-10% CH_2Cl_2:MeOH) to yield the desired product (214 mg,0.91 mmol, 91%). The spectral data for this compound matches with the literature report.[4]

***tert*-Butyl(2-oxo2-((6-(6-pyridin-2-yl)-1,2,4,5-tetrazin-3-yl)amino)ethyl) carbamate (10b):**



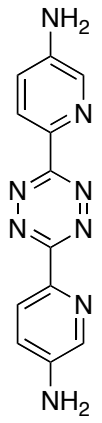
The general procedure for oxidation was followed using tert-butyl (2-oxo-2-((6-(6-(pyridin-2-yl)-1,4-dihydro-1,2,4,5-tetrazin-3-yl)pyridin-3-yl)amino)ethyl)carbamate[5] (410 mg, 1.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-10% CH_2Cl_2:MeOH) to yield the desired product (337 mg, 0.83 mmol, 83%). The spectral data for this compound matches with the literature report.[5]

3,6-Di(furan-2-yl)-1,2,4,5-tetrazine (10c):



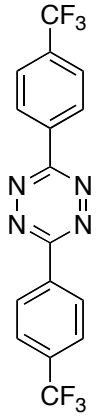
The general procedure for oxidation for followed using 3,6-di(furan-2-yl)-1,4-dihydro-1,2,4,5-tetrazine[6] (216 mg, 1.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-100% hexanes:CH_2Cl_2) to yield the desired product (187 mg, 0.88 mmol, 88%). The spectral data for this compound matches with the literature report.[7]

6,6'-(1,2,4,5-Tetrazine-3,6-diyl)bis(pyridin-3-amine) (10d):



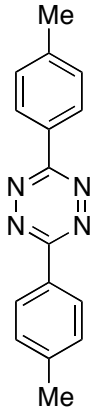
The general procedure for oxidation was followed using 6,6'-(1,4-dihydro-1,2,4,5-tetrazine-3,6-diyl)bis(pyridin-3-amine)[8] (268 mg, 1.0 mmol). The reaction mixture was diluted with CH_2Cl_2 (8 mL), centrifuged, and the supernatant was decanted. The residue was thrice suspended with CH_2Cl_2 (8 mL), sonicated, centrifuged and decanted. The solid was dried under vacuum to yield the desired product (261 mg, 0.98 mmol, 98%). The spectral data for this compound matches with the literature report.[8]

3,6-Bis(4-trifluoromethyl)phenyl)-1,2,4,5-tetrazine (10e):



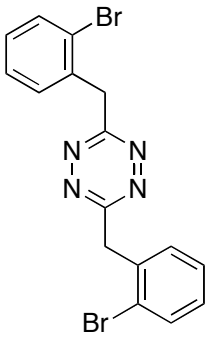
The general procedure for oxidation was followed using 1 mmol of **9e** (438 mg of material of 83% purity, *vide supra*). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-100% hexanes:CH_2Cl_2) to yield the desired product (353 mg, 0.95 mmol 95%) mp: 273-276 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.75 (d, *J* = 8.2, 4H), 7.84 (d, J = 8.2, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 163.5 (u), 134.8 (u), 134.5 (u) [q, ^{2}J(CF) = 33 Hz], 128.5 (dn), 126.4 (dn) [q, ^{3}J(CF) = 3.7 Hz], 123.6 (u) [q, ^{1}J(CF) = 273 Hz]; IR (neat, KBr, cm^{-1}): 2930, 1420, 1396, 1316, 1109, 853; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd for $C_{16}H_8F_6N_4^+$ 370.0648; found 370.0663.

3,6-Di-*p*-tolyl-1,2,4,5-tetrazine (10f):



The general procedure for oxidation was followed using 1 mmol of **9f** (318 mg of material of 83% purity, *vide supra*). The reaction mixture was adsorbed on silica gel and purified by column chromatography (0-50% hexanes:CH_2Cl_2) to yield the desired product (201 mg, 0.77 mmol, 77%**)** as a pink solid.. The spectral data for this compound matches with the literature report.[7]

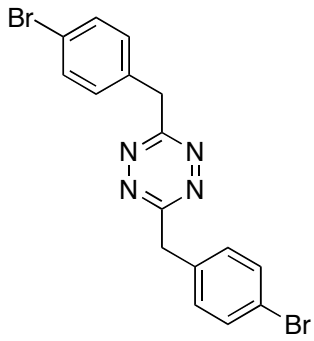
3,6-Bis(2-bromobenzyl)-1,2,4,5-tetrazine (10g):



The general procedure for oxidation was followed using **9g** (422 mg, 1.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (0-50% hexanes:CH_2Cl_2) to yield the desired product (359 mg, 0.83 mmol, 83%**)** as a pink solid, mp: 154-156 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.59 (dd, *J* = 8.1, 1.4 Hz, 2H), 7.42 (dd, J = 7.8, 1.7 Hz, 2H), 7.32 (td, J = 7.8, 1.4 Hz, 2H), 7.18 (td, J = 8.1, 1.7 Hz, 2H), 4.83 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.5 (u), 135.4 (u), 133.1 (dn), 131.9 (dn), 129.2 (dn), 127.8 (dn), 124.9 (u), 41.4 (u); . IR (neat, KBr, cm^{-1}): 3052, 1469, 1388, 1338, 1298, 1239, 1232, 1086, 1044, 892, 757; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd for $C_{16}H_{12}Br_2N_4^+$ 419.9403; found 419.9432.

Alternatively the general procedure for tetrazine synthesis without purification of the intermediate dihydrotetrazine was followed using 2-(2-bromophenyl)acetonitrile (392 mg, 2.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-50% hexanes:CH_2Cl_2) to yield the desired product (166 mg, 0.40 mmol, 40%).

3,6-Bis(4-bromobenzyl)-1,2,4,5-tetrazine (10h):



The general procedure for oxidation was followed using **9h** (422 mg, 1.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0–50% hexanes:CH_2Cl_2) to yield the desired product (317 mg, 0.75 mmol, 75%) as a pink solid. mp:149 - 152 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.44 (d, J = 8.4, 4H), 7.29 (d, J = 8.3, 4H), 4.55 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.9 (u), 134.6 (u), 132.1 (dn), 131.0 (dn), 121.6 (u), 40.6 (u); IR (neat, KBr, cm^{-1}): 3082, 2931, 1487, 1377, 1324, 1139, 1068, 1010, 649; HRMS (LIFDI-TOF) *m/z*: $[M]^+$ calcd for $C_{16}H_{12}Br_2N_4^+$ 419.9403; found 419.9410

Alternatively the general procedure for tetrazine synthesis without purification of the intermediate dihydrotetrazine was followed using 2-(4-bromophenyl)acetonitrile (392 mg, 2.0 mmol). The reaction mixture was adsorbed on silica gel and purified by column chromatography (gradient 0-50% hexanes:CH_2Cl_2) to yield the desired product (164 mg, 0.39 mmol, 39%).

References

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