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Preparation of 8-(Arylsulfanyl)adenines with Diazonium Salts under Mild, Aerobic Conditions

Marco A. Biamonte,* Jiandong Shi, David Hurst, Kevin Hong, Marcus F. Boehm, and Srinivas R. Kasibhatla

Conforma Therapeutics Corporation, 9393 Towne Centre Drive, Suite 240, San Diego, California 92121

mbiamonte@conformacorp.com

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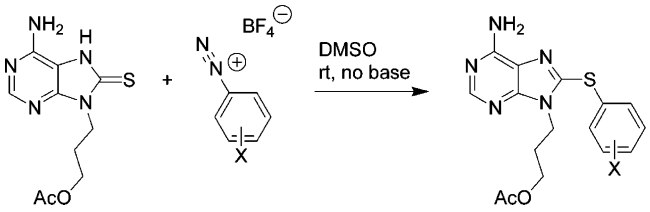

8-(Arylsulfanyl)adenines **11** were prepared in up to 75% yield by reacting the 8-thionoadenine **6** (acetic acid 3-(6-amino-8-thioxo-7,8-dihydropurin-9-yl)propyl ester) with benzenediazonium tetrafluoroborates in DMSO. Benzenediazonium ions carrying an electron-withdrawing substituent gave the highest yields. The reaction proceeded smoothly at room temperature without any base and could be performed under air atmosphere. The extremely mild conditions are compatible with a wide range of functional groups.

Heat Shock Protein 90 (Hsp90) is a molecular chaperone that maintains the proper conformation of "client" proteins.[1] Hsp90 client proteins include three clinically validated cancer targets, Her-2/neu (Herceptin), Bcr-Abl (Gleevec), and the androgen receptor (Casodex), as well as several oncogenes, such as EGFR, MET, IGF-1R, Akt, Raf-1, and p53. Mutant client proteins, as found in cancer cells, are particularly dependent on Hsp90 to preserve their conformation and function. Inhibition of Hsp90 causes these client proteins to adopt aberrant conformations, which is followed by their rapid degradation by the proteasome, resulting in cell-cycle arrest and ultimately cell death. Hence, Hsp90 inhibitors can prevent tumor growth by damaging multiple proliferative pathways. Moreover, Hsp90 occurs in an activated form in cancer cells and in a latent form in normal cells. This provides a rare opportunity for medicinal chemists to specifically target cancer cells with inhibitors selective for the activated form.[1c] This concept has been demonstrated in murine models (tumor xenografts) and is currently being proved in phase I/II clinical trials using the semisynthetic inhibitor 17-allyl-17-desmethoxygeldanamycin.

As derivatives of the structurally complex natural product geldanamycin are expensive, often difficult to formulate, and limited to parenteral use, we are developing orally active, small-molecule inhibitors of Hsp90. Recently, we and others have identified 8-arylsulfanyl purines[2] and 8-benzyl purines[2,3] as new classes of potent and specific Hsp90 inhibitors.[4] For our investigations, the need arose to produce 8-(arylsulfanyl)adenines having the generic structure **5**, which we initially obtained by reacting 8-bromoadenine (**1**) with thiophenolates in DMF at 70−120 °C (Scheme 1, eq 1).

This method had several limitations: (i) some thiophenols did not withstand the reaction temperatures, (ii) the preparation of 8-bromoadenine **1** by bromination of adenine[5] was in our hands capricious and often incomplete, and (iii) the range of commercial thiophenols did not have the substitution pattern relevant to us.

We therefore evaluated an alternative route by reacting 6-amino-7,9-dihydropurine-8-thione (**3**) with diazonium salts (Scheme 1, eq 2). The sulfur atom is present on the adenine ring rather than on the benzene ring. This formally reverses the polarity of the reaction, and the adenine becomes the nucleophile.

At the outset of our work, little was known about the reaction of diazonium salts with *heterocyclic* sulfur nucleophiles. In fact, only one example was reported:[6] 2,4,6-trimethylbenzenediazonium chloride was reacted with the KOH salt of 3-mercapto-1*H*-1,2,4-triazole to yield the corresponding sulfide (MeOH, 0−9 °C, 69%).

In contrast, the reaction of diazonium salts with structurally simpler sulfur nucleophiles, such as HS^-, RS^-, or ArS^-, is well precedented.[7] When the sulfur nucleophile is HS^- or an equivalent thereof, the reaction is a standard route to thiophenols.[8,9] The diazonium salts are then typically reacted with $ROCS_2^-$ (Leuckart synthesis),[10] S_n^{2-},[8] NCS^-,[8] $S_2O_3^{2-}$,[8] or thiourea[11] to give, respectively, xanthates, thiophenolates, isothiocyanates,

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SCHEME 1

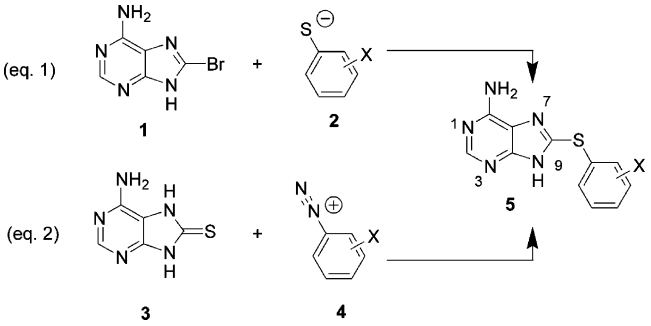

TABLE 1. Reaction of 3 with Diazonium Salts[a]

entry	substituent on **4**	conditions	unreacted **3** (%)	yield of **5** (%)	yield of **5** based on unreacted **3** (%)
1	4-NO_2	rt, 1 h	15	41	49
2	4-F	rt, 12 h	41	33	56
3	3-OMe	rt, 1 h	58	31	74
4	4-Me	60 °C, 5 h	71	7	25

[a] All reactions were performed in DMF, at a concentration of 1 M, with 1.2 equiv of diazonium salt. Yields were determined by HPLC and are corrected for the differences in absorbance.

Bunté salts, and isothiuronium salts which can all be hydrolyzed with alkali to provide a thiophenol. When the nucleophile is RS^-, the reaction is equally feasible and has been reported with EtSNa (H_2O, 70 °C),[12] straight-chain or branched alkanethiolates RSNa (MeOH, 25 °C), MeSCu (H_2O, 4 °C),[13] F_3C-SCu (CH_3CN, 50 °C),[14] and $HS-CH_2COOH$ (no base, H_2O, 100 °C),[15] Finally, the reaction of diazonium salts with ArS^- has been reported several times,[16] usually with PhSNa (DMSO[16] or MeOH,[17] 25 °C).

While our work was in progress, an interesting Cu-mediated coupling of **3** with iodobenzenes was published,[18] and both methods will be compared in the conclusion of this paper.

In a series of preliminary experiments, we were gratified to observe that a suspension of the thione **3** in DMF reacted cleanly with several diazonium salts (Table 1). Electron-withdrawing substituents (NO_2, F; entries 1,2) gave the highest yields. The reaction was fastest with either the electron-withdrawing NO_2 or the electron-donating MeO. It was slower with F, and very slow with methyl. Unfortunately, the low solubility of **3** consistently resulted in low conversions, yielding 15−71% of unreacted starting material which could be selectively removed by an aqueous workup.

We therefore shifted our focus to the thionopurine **6**

SCHEME 2

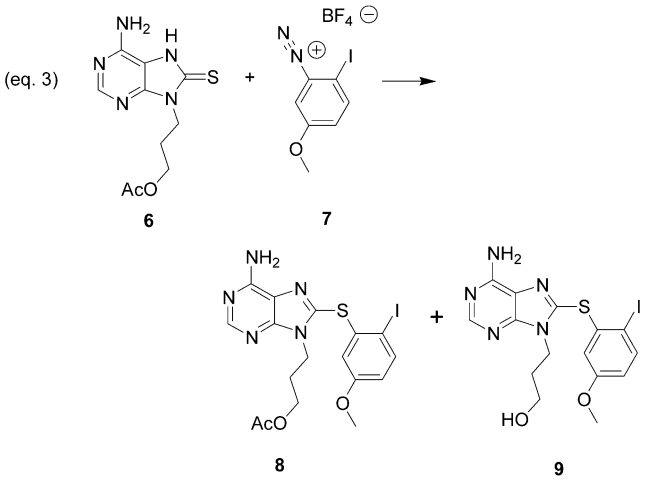

TABLE 2. Solvent Optimization[a]

entry	solvent	unreacted **6** (%)	yield of **8** (%)	yield of **9** (%)
1	DMSO	22	35	4
2	Acetone	31	35	7
3	CH_3CN	9	34	9
4	MeOH	27	27	4
5	DMF	58	17	2
6	i-PrOH	57	13	1
7	THF	40	4	1

[a] All reactions were performed at a concentration of 0.1 M, with 1.0 equiv of NaOH and 1.2 equiv of **7**, rt, 1 h. Yields were determined by HPLC and are corrected for the differences in absorbance.

TABLE 3. Base Optimization[a]

entry	base	unreacted **6** (%)	yield of **8** (%)	yield of **9** (%)
1	no base, 0.5 M	4	56	0
2	pyridine	22	48	0
3	$NaHCO_3$	18	46	0
4	no base, 0.1 M	19	45	1
5	Cs_2CO_3	20	45	3
6	*i*-Pr_2NEt	31	39	2
7	*t*-BuOK	19	36	8
8	NaOH	17	33	5
9	DBU	21	32	3

[a] All reactions were performed in DMSO, at a concentration of 0.1 M unless otherwise noted, with 1.0 equiv of base and 1.2 equiv of **7**, rt, 1 h. Yields were determined by HPLC and are corrected for the differences in absorbance.

(Scheme 2),[19] in which the N(9) side chain significantly increased the solubility and further provided a functional group (AcO) suitable for our medicinal chemistry efforts. First, we optimized the solvent using **7** as the diazonium salt and aqueous 1 M NaOH as the base (Table 2). No precautions were taken to exclude moisture or air, and in all cases the reaction gave the desired product **8** and the deacetylated product **9**. The best yield of **8** and ratio of **8**/**9** was obtained in DMSO, although acetone and CH_3CN were also acceptable solvents.

Next, we optimized the base (Table 3) using DMSO as the solvent,[20] and we were surprised to observe that the

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(19) For the preparation of **6**, see Supporting Information.
(20) In the absence of a strong base, **6** is only sparingly soluble in acetone or acetonitrile.

SCHEME 3

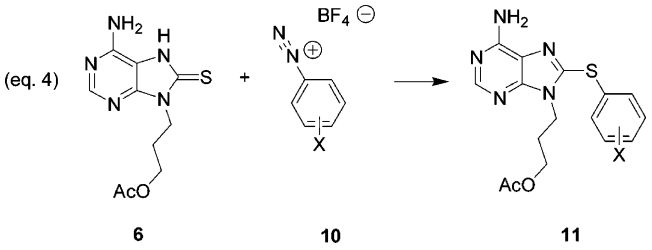


TABLE 4. Preparation of 8-Sulfanyladenine 11

entry	X	unreacted **6** (%)	yield of **11** (%)
1	4-Br	4	75
2	4-NO_2	7	71
3	4-F	17	61
4	4-CO_2Et	0	61
5	5-MeO-2-I	3	56
6	2,4-di-F	0	38
7	4-MeO	57	27
8	4-Me	39	23
9	H	6	21
10	3-MeO	15	21
11	4-Et_2N	72	<10

[a] All reactions were carried out in DMSO at a concentration of 0.5 M, with 1.2 equiv of diazonium salt, rt, 24 h. Yields were determined by HPLC and are corrected for the differences in absorbance.

reaction proceeded best in the absence of any base. With few exceptions,[15] thiols require deprotonation to react with diazonium salts. Thus, it is remarkable that a neutral thiol species can react so fast with a diazonium salt at room temperature. A high concentration (0.5 M) gave the optimal yield of **8**, and importantly, under these conditions no deacetylation was observed.

With these optimized conditions in hand (DMSO, no base, 0.5 M), we screened a range of diazonium salts and obtained yields as high as 75% (Scheme 3, Table 4).[21] The reaction proceeded favorably with benzenediazonium salts carrying electron-withdrawing substituents (F, Br, NO_2, CO_2Et), while electron-donating substituents (Me, MeO, Et_2N) resulted in poorer yield. The 2,4-difluorobenzenediazonium ion (entry 6) was presumably too reactive to result in a clean reaction. To confirm that the yields determined by HPLC were reliable, two representative compounds were isolated by flash chromatography. The 4-nitro derivative (entry 2) and the 2-iodo-5-methoxybenzene derivative (entry 5) were isolated in 64% and 51% yield, respectively, which is comparable with the HPLC yields. Their structure was confirmed by ^{1}H NMR, ^{13}C NMR, CHN, and/or HRMS. In general, these reactions did not give one major byproduct, but rather an array of minor impurities, some of which were bright orange, and occasionally complicated the chromatographic separations.

The kinetics of the reactions were monitored by HPLC, and Figure 1 depicts the results for 4-(EtO_2C)$C_6H_4N_2^+$-BF_4^-, a moderately reactive diazonium salt. Addition of the diazonium salt to the reaction mixture was characterized by a rapid evolution of N_2 which subsided after 1−2 min. After 5 min, 41% of the starting material **6** was consumed, producing 31% of the 8-sulfanylpurine **11**. After this initial phase, the reaction rate decreased significantly, yielding 50% of **6** after 40 min. The starting material and the diazonium salt were consumed at a constant rate, while the amount of product **11** reached a plateau at 61%. Thereafter, impurities appeared suggesting that **11** can itself react to some extent with the diazonium cation.

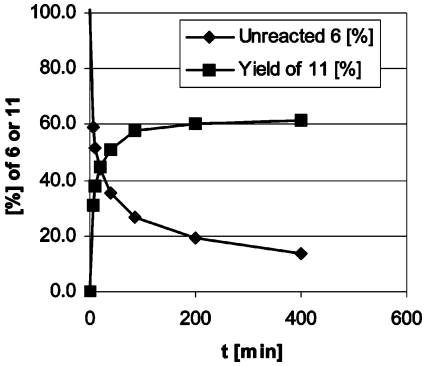
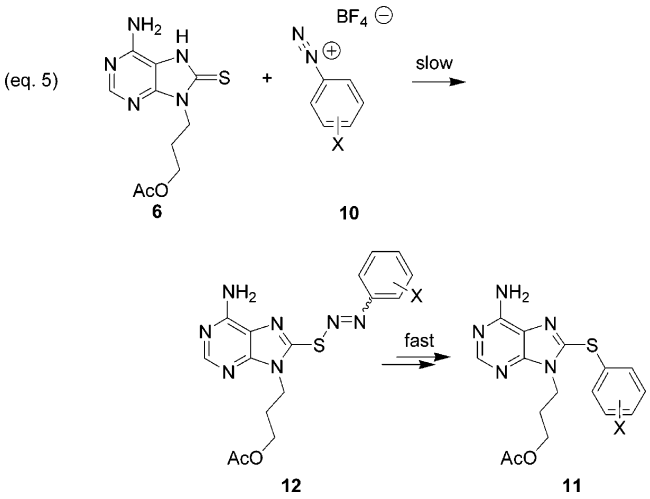


FIGURE 1. Concentration of starting material **6** and product **11** as a function of time.

SCHEME 4

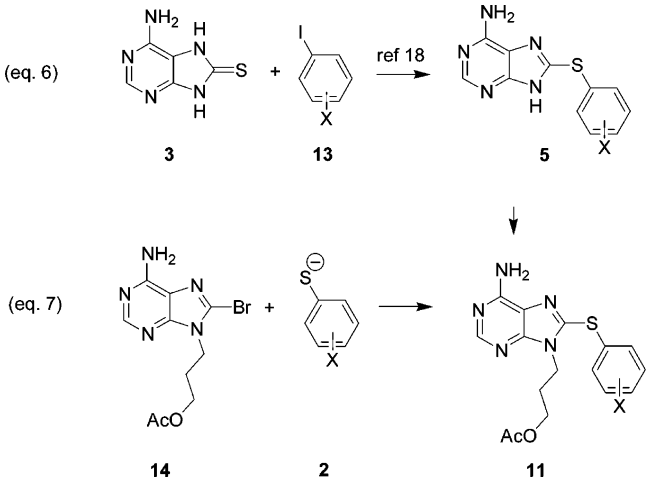


It is tempting to postulate that the reaction mechanism involves the intermediate diazosulfide **12** (Scheme 4) which would rapidly decompose by a series of radical- and single-electron-transfer reactions with loss of dinitrogen. Analogous disulfides have been postulated for the reaction of PhSNa with ArN_2^+.[16] They are also known to accumulate in the reaction of EtSNa with PhN_2^+.[22] Furthermore, the 8-thionopurine **6** may be regarded as a substituted thiourea, and the reaction of thiourea with the *p*-nitrobenzenediazonium ion gives a diazosulfide which could be isolated.[11] In our case, if a diazosulfide is indeed in an intermediate, it must decompose faster than it forms because no intermediates were detected by HPLC.

(21) **General Procedure.** The thione **6** (400 mg, 1.5 mmol) was dissolved in DMSO (3.0 mL), and the appropriate benzenediazonium tetrafluoroborate (1.2 equiv) was added in 10 small portions, waiting for the N_2 evolution to cease before adding the next portion (total addition time: 2−5 min). The reaction was stirred at rt for 24 h whereupon it turned dark orange. Workup (EtOAc/water), washing (brine), and drying (Na_2SO_4) gave the crude material which was purified by preparative TLC (EtOAc/hexane) or flash chromatography (2−4% MeOH in CH_2Cl_2, then EtOAc).

(22) The reaction of EtSNa with PhN_2^+ gives EtSN=NPh, which breaks down at 70 °C to give EtSPh (see ref 12).

SCHEME 5



This mechanism, however, does not account for the kinetics reported in Figure 1. One would expect the reaction to be first order in each reactant (**6** and **11**), i.e., overall second order. However, further data analysis suggests that the reaction is overall *third* order.[23] This implies a more complex reaction mechanism, or perhaps the coexistence of two different mechanisms, but their nature is not apparent to us without additional investigations.

In conclusion, the reaction of 8-thionoadenine **6** in the presence of benzenediazonium salts is a remarkably facile reaction. This method readily allows the rapid generation of 8-(phenylsulfanyl)adenines **11**, under neutral conditions and at room temperature. There is no need to exclude air or moisture, and oxidation of the thionopurine **3** or **6** to the corresponding disulfide was never observed. This method is best suited for adenines that are substituted at N(9), because they are more soluble, and for diazonium ions that carry electron-withdrawing substituents. This method also allows for the introduction of halogens (Br, I) which can be further utilized for Pd-mediated functionalization.

There are two alternative routes to compounds of structure **11**. The first one consists of coupling the thionoadenine **3** with an iodobenzene under Cu-catalysis[18] to give **5**, which is then alkylated (Scheme 5, eq 6). The Cu-catalyzed coupling is certainly the method of choice for the generation of N(9)-unsubstituted adenines, in part because the conditions (*t*-BuONa, DMF, 110 °C, 24 h) facilitate the dissolution of the starting material **3**. Also, the Cu-catalyzed coupling is not limited to electron-withdrawing substituents. However, the reaction does require rigorous exclusion of air, and the strongly basic conditions preclude the use of some functional and/or protective groups, such as esters. Furthermore, the alkylation of **5** typically gives a 2:1 mixture of N(9)- and N(3)-alkylated regioisomers which are often difficult to separate by silica gel chromatography. It is therefore more convenient to introduce the substituent early in the synthetic scheme.[24]

The alternative to generate compounds of structure **11** consists of coupling the 8-bromoadenine **14** with thiophenolates (Scheme 5, eq 7, K_2CO_3, DMF, 70−100 °C). The N(9) substituent provides the solubility so critically lacking in 8-bromoadenine (**1**) and renders the reaction perfectly satisfactory in many cases, even if some of the thiophenol inevitably oxidizes to the corresponding disulfide. This may be the reaction of choice if the thiophenol is commercially available, but is less than optimal if the thiophenol requires preparation. Thiophenols can be difficult to prepare, and their purification can be onerous because of their tendency to oxidize. Their stench further renders distillations and chromatographies notoriously disagreeable. In contrast, diazonium tetrafluoroborates are odorless, solid (often crystalline), and easily generated from anilines, for which the commercial pool is comparatively much larger and cheaper than for thiophenols.

The three methods described in this paragraph are clearly complementary, and the interplay of factors mentioned in this chapter will guide the chemist in selecting the method most appropriate to their application.

Acknowledgment. We thank Professors S. Danishefsky and D. Boger for their encouragement.

Supporting Information Available: General procedure for the preparation of diazonium tetrafluoroborates. Preparation of thiones **3** and **6**. ^{1}H NMR data for compounds listed in Table 1. ^{1}H NMR and HRMS data for compounds listed in Table 4. Analysis of second- vs third-order kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) A plot of $[\mathbf{6}]^{-1}$ versus time is not linear, excluding the possibility of an overall second-order reaction, but a plot of $[\mathbf{6}]^{-2}$ versus time is linear, which is consistent with a third-order reaction (see the Supporting Information).

(24) The alkylation of adenine provides mostly the N(9) isomer, and this property was used to prepare **6** (see the Supporting Information). The Cu-catalyzed method is presumably also applicable to N(9)-substituted 8-thionoadenines; the authors did not comment on this possibility.