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Synthesis of Deuterium-Labelled 2,5-Bis(4-amidinophenyl)furan, 2,5-Bis[4-(methoxyamidino)phenyl]furan, and 2,7-Diamidinocarbazole

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Summary

The syntheses of 2,5-bis(4-amidinophenyl)furan-d_8 (**1-d_8**) and 2,5-bis[4-(methoxyamidino)phenyl]furan-d_8 (**2-d_8**) from bromobenzene-d_5 in six steps, and of 2,7-diamidinocarbazole-d_6 (**3-d_6**) from biphenyl-d_{10} in five steps, are described.

Key Words: deuterium-labelled, 2,5-bis(4-amidinophenyl)furan-d_8, 2,5-bis[4-(methoxy-amidino)phenyl]furan-d_8, 2,7-diamidinocarbazole-d_6, antimicrobial, prodrug

Introduction

The aryl diamidines 2,5-bis(4-amidinophenyl)furan (**1**) and 2,7-diamidinocarbazole (**3**) have been found to have broad spectrum antimicrobial activity. Previously **1** was reported to be effective against *Trypanosoma rhodesiense* in mice (1). Diamidines **1** and **3** exhibited excellent activity versus *Pneumocystis carinii* pneumonia (PCP) in an immunosuppressed rat model when administered intravenously, but they were ineffective when given orally (2-4). However, the diamidines **1** and **3** were effective when given orally in a neonatal mouse model for *Cryptosporidium parvum* (5, 6). Recent studies have shown that 2,5-bis[4-(methoxyamidino)phenyl]furan (**2**) functioned as a

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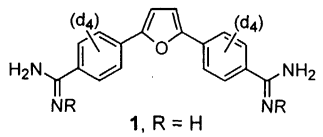
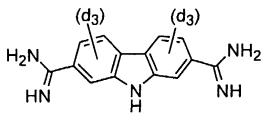
prodrug of **1**, as the amidoxime was found to be active when administered orally in the immunosuppressed rat model for PCP (7). In view of the efficacy of **1-3** against these pathogens, preclinical toxicity and metabolism studies are in progress in anticipation of Phase I clinical trials of these compounds. The novel isotopically labelled compounds **1-d_8**, **2-d_8**, and **3-d_6** are required as analytical (MS) internal standards for these pre-clinical studies. Here we report their syntheses.



1, R = H
2, R = OCH_3



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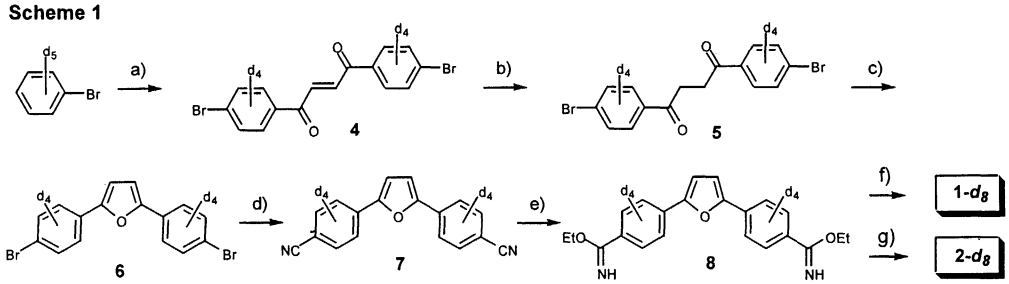
Results and Discussion

The preparation of the title compounds **1-d_8** and **2-d_8** involved the common intermediate 2,5-bis(4-cyanophenyl)furan-d_8 (**7**, Scheme 1). Attractive routes to **7** involved either 1) the synthesis of the cyano-substituted 1,4-diketone using Stetter methodology (8, 9), followed by cyclodehydration in the presence of an acid catalyst; or 2) a direct synthesis by the Stille coupling of 2,5-bis(tributylstannyl)furan and 4-bromobenzonitrile (10). However, these routes would require either deuterium-labelled 4-cyanobenzaldehyde or 4-bromobenzonitrile, which were not commercially available. Although the originally described pathway to unlabelled **7** involves additional steps (1), this approach was most expedient for the preparation of labelled compound due to the commercial availability of bromobenzne-d_5.

Scheme 1



a) fumaryl chloride, $AlCl_3$, CS_2 b) $SnCl_2$, HOAc, EtOH c) H_2SO_4, Ac_2O d) CuCN, DMF e) HCl, EtOH f) NH_3, EtOH g) CH_3ONH_2, EtOH

The reaction of bromobenzene-d_5 with 0.5 equivalents of fumaryl chloride in the presence of $AlCl_3$ gave dibenzoylethylene **4** in 46% yield. No loss of deuterium under these strongly Lewis acidic conditions was evident by ^1H NMR or mass spectrometry. Subsequent attempts to reduce the double bond of both **4** and its unlabelled analog by the standard Zn/AcOH method gave a mixture of **5** and an unidentified impurity. However, olefin **4** was reduced quickly and cleanly to **5** (80% yield) using $SnCl_2$ in a refluxing mixture of AcOH/EtOH (1:1). The choice of $SnCl_2$ was adapted from its use in combination with HCl to effect a direct furanization of a dibenzoylethylene derivative (11). The isolated diketone **5** underwent a separate cyclodehydration (in 76% yield) to furan **6** using Ac_2O/H_2SO_4. The reaction of dibromo intermediate **6** with CuCN in refluxing DMF gave dinitrile **7** in 66% yield. The diimidate **8** was prepared from dinitrile **7** using standard Pinner methodology. Despite the exposure of **7** to saturated ethanolic HCl for 2 weeks, no loss of deuterium was evident in the ^1H NMR spectrum of **8**. The reaction of **8** with ammonia or methoxylamine in anhydrous ethanol gave target compounds **1-d_8** and **2-d_8** in 93% and 63% yields, respectively.

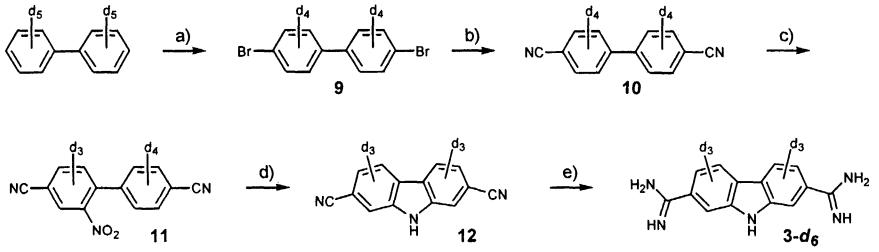
The preparation of carbazole **3** from 2,5-dibromonitrobenzene via 2,7-dibromocarbazole (12) has been previously described (3). This pathway involved a symmetrical Ullmann coupling to give 4,4'-dibromo-2,2'-dinitrobiphenyl, reduction of the two nitro groups using Sn/HCl or $SnCl_2$, followed by treatment of the diamine with phosphoric acid at 200 °C to give 2,7-dibromocarbazole. The reaction of the latter with copper (I) cyanide in refluxing DMF gave the corresponding dinitrile, which was converted to **3** using standard Pinner methodology.

The synthesis of **3-d_6** by the analogous methodology required 2,5-dibromonitrobenzene-d_3, which was readily prepared by the nitration of commercial 1,4-dibromobenzene-d_4 under standard conditions. The remaining steps of the synthesis were performed, but several problems were encountered. The Ullmann reaction proceeded more slowly than the analogous reaction using unlabelled material, and the additional step of column chromatography was necessary to purify the product. This reaction, as well as the ring closure and cyanation reactions, gave significantly lower yields than the analogous reactions using unlabelled material. The nitro groups were reduced

more effectively with Sn/HCl than with $SnCl_2$. Most significantly, it was necessary to use deuterium-labelled acids in both the nitro reduction and ring closure steps to prevent loss of the deuterium label.

An alternate synthesis of **3-d_6** from biphenyl-d_{10} in five steps is shown in Scheme 2. This pathway provided the advantages of a less expensive and more advanced starting material, and no requirement for additional deuterated reagents. This pathway began with the bromination of biphenyl-d_{10} using bromine vapor to give dibromobiphenyl **9** in 39% yield (13). A higher yield could be obtained by repeating the bromination (Br_2/HOAc) on the monobromo product recovered from the mother liquors. It was envisioned that 2,7-dibromocarbazole-d_6 could be prepared in two steps by the nitration of **9**, followed by a Cadogan ring closure (3, 14) of the resulting 2-nitrobiphenyl. However, the nitration of unlabelled **9** under several conditions gave a mixture of the 2-nitro and 2,3'-dinitro products with a predominance of the latter. Reversal of the order of steps led to a successful synthesis. The reaction of **9** with copper(I) cyanide in refluxing DMF gave the dinitrile **10** in 98% yield. The nitration of **10** was complete within one hour and gave the 2-nitro biphenyl **11** in 47% yield. A ring closure of unlabelled **11** under standard Cadogan conditions (refluxing neat triethyl phosphite) gave no unlabelled **12**, but 2,7-dicyano-9-ethylcarbazole in 22% yield. Labelled carbazole **12** was prepared in 42% yield by the reaction of **11** with triisopropyl phosphite in refluxing toluene, with no significant amount of purported *N*-alkylation product detected by HPLC. Finally, **3-d_6** was prepared from **12** in 47% yield using standard Pinner methodology (3).

Scheme 2



a) Br_2 (vapor) b) CuCN, DMF c) HNO_3, H_2SO_4 d) triisopropyl phosphite, toluene e) EtOH, HCl, 1,4-dioxane, then NH_3, EtOH

Conclusion

An effective synthesis of 2,5-bis(4-amidinophenyl)furan-d_8 (**1-d_8**) and 2,5-bis[4-(methoxyamidino)phenyl]furan-d_8 (**2-d_8**) starting from bromobenzene-d_5 has been developed without detectable loss of the deuterium label. The overall yields were 16% and 9%, respectively. Two syntheses of 2,7-diamidinocarbazole-d_6 (**3-d_6**) have been developed, also without detectable loss of the deuterium label. Overall yields from the pathways starting from 1,4-dibromobenzene-d_4 and biphenyl-d_{10} were 9% and 5%, respectively. In effect, a new synthesis of unlabelled **3** from 4,4'-dibromobiphenyl has been developed. The use of these compounds as analytical (MS) internal standards for metabolism and pharmacokinetic studies will be described in due course.

Experimental

Bromobenzene-d_5 (99.5 atom % D, lot # 08162MS) was purchased from Aldrich Chemical Company. 1,4-Dibromobenzene-d_4 (98 atom % D, lot # P-9278) and biphenyl-d_{10} (99 atom % D, lot # P-3686) were purchased from Cambridge Isotope Labs. Anhydrous EtOH was distilled under nitrogen from $Mg(OEt)_2$ and stored under nitrogen. Other reagents and solvents were purchased and used as received. NMR spectra were recorded on a Varian 300 or 400 MHz instrument, with reported values being relative to TMS. IR spectra were obtained using a Perkin Elmer Spectrum One reflectance spectrophotometer (KBr matrix). MS analyses were performed by the MS-laboratory of the Georgia Institute of Technology using a 70-SE instrument, or at UNC-Chapel Hill using a Micromass Quattro II instrument. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. HPLC analyses were recorded on a Hewlett-Packard 1090 Series II chromatograph using a Zorbax SB C8 column (4.6 x 75 mm, 3.5 micron) and UV dectection (254 or 265 nm). Mobile phases A and B each contained 80 mM formic acid, 20 mM ammonium formate, and 15 mM triethylamine in either water or 75% CH_3CN, respectively. In Method A, the percentage of mobile phase B was increased from 0 to 30% in 6 minutes, from 30 to 75% in 4 minutes, and maintained for 2 minutes. In Method B, the percentage of mobile phase B was increased from 60 to 75% in 10 minutes.

***trans*-1,2-Bis(4-bromobenzoyl)ethene-d_8 (4).** Bromobenzene-d_5 (11.34 g, 70 mmol) was added to a stirred suspension of aluminum chloride (15.7 g, 118 mmol) in anhydrous carbon disulfide (35 mL) in a flask equipped with a condenser and a drying tube. Fumaryl chloride (5.35 g, 35 mmol) was added in portions, and the mixture was heated in an oil bath at ~50-55 °C for 2 days. The dark red reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The extracts were washed with brine, dried over Na_2SO_4, concentrated to near dryness, and diluted with petroleum ether. The resulting precipitate was filtered off and rinsed with a small amount of ether to give a golden orange crystalline solid (6.85 g). The solid was suspended in boiling CH_2Cl_2 (150 mL). The mixture was concentrated to near dryness, then diluted with excess ether. The product was filtered off to give a yellow crystalline solid (6.50 g, 46%): mp 190-191.5 °C (lit (15) 188.5 °C for unlabelled compound); IR ν 1645 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 7.86 (s, 2 H); EIMS *m/z* (relative intensity) 404 (13.1, $C_{16}H_2D_8{}^{81}Br_2O_2$), 402 (24.6, $C_{16}H_2D_8{}^{81}Br^{79}BrO_2$), 400 (12.9, $C_{16}H_2D_8{}^{79}Br_2O_2$); high resolution EIMS calcd for $C_{16}H_2D_8{}^{79}Br_2O_2$ *m/z* 399.9550, found 399.9590.

1,2-Bis(4-bromobenzoyl)ethane-d_8 (5). $SnCl_2$ dihydrate (6.50 g, 28.8 mmol) was added to a suspension of **4** (3.25 g, 8.08 mmol) in a mixture of AcOH (110 mL) and absolute EtOH (110 mL) at ambient temperature. The mixture was heated to reflux over ~10 min and maintained for 5-10 min, at which point the mixture had become a pale-yellow solution. TLC ($CHCl_3$) showed a complete and clean reduction. The solution was concentrated to ~150 mL and allowed to stand at room temperature for 1 h. The solid was filtered off and rinsed with ether to give white crystals (2.60 g, 80%): mp 183-184 °C (lit (16) 182 °C and (17) 183-185 °C for unlabelled compound); IR ν 1672 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ 3.38 (s, 4 H); EIMS *m/z* (relative intensity) 406 (4.5, $C_{16}H_4D_8{}^{81}Br_2O_2$), 404 (9.7, $C_{16}H_4D_8{}^{81}Br^{79}BrO_2$), 402 (5.7, $C_{16}H_4D_8{}^{79}Br_2O_2$); high resolution EIMS calcd for $C_{16}H_4D_8{}^{79}Br_2O_2$ *m/z* 401.9706, found 401.9707.

2,5-Bis(4-bromophenyl)furan-d_8 (6). A solution of H_2SO_4 (3 drops by Pasteur pipet) in Ac_2O (1 mL) was added through the condenser to a gently refluxing solution of **5** (3.60 g, 8.91 mmol) in Ac_2O (32 mL). A mild exotherm resulted. After 4-5 min, the solution was allowed to cool slowly for 1 h, and the solid was filtered off and rinsed

with hexanes to give colorless needles (2.60 g, 76%): mp 205-205.5 °C (lit (18) 206.5-208 °C for unlabelled compound); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (s, 2 H). EIMS *m/z* (relative intensity) 388 (60.0, $C_{16}H_2D_8{}^{81}Br_2O$), 386 (100, $C_{16}H_2D_8{}^{81}Br^{79}BrO$), 384 (53.4, $C_{16}H_2D_8{}^{79}Br_2O$); high resolution EIMS calcd for $C_{16}H_2D_8{}^{79}Br_2O$ *m/z* 383.9601, found 383.9699.

2,5-Bis(4-cyanophenyl)furan-d_8 (7). A suspension of **6** (3.40 g, 8.81 mmol) and CuCN (3.0 g, 33.5 mmol) in anhydrous DMF (50 mL) was heated at reflux under nitrogen for 21 h. The cooled reaction mixture was poured into a mixture of water (100 mL) and conc. ammonium hydroxide (50 mL) and extracted with $CHCl_3$ (~300 mL total). The extract was washed twice with water, with brine, dried (Na_2SO_4), and evaporated. The residue was diluted with a small amount of MeOH, and a golden yellow solid was filtered off. The product (1.88 g) was purified by column chromatography over silica ($CHCl_3$) to give a fine yellow crystalline solid. This material was suspended in a small volume of boiling EtOH and filtered off immediately to give a yellow solid (1.62 g, 66%): mp 292-293 °C (lit (1) 294-295 °C for unlabelled compound); IR ν 2222 cm^{-1}, ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.41 (s, 2 H); EIMS *m/z* (relative intensity) 278 (100, $C_{18}H_2D_8N_2O$); high resolution EIMS calcd for $C_{18}H_2D_8N_2O$ *m/z* 278.1295, found 278.1292.

2,5-Bis(4-ethoxyiminoylphenyl)furan-d_8 dihydrochloride (8). A stirred suspension of **7** (1.60 g, 5.75 mmol) in anhydrous EtOH (80 mL) cooled in an ice-water bath was saturated with dry HCl and then sealed. The mixture was stirred at ambient temperature for 2 weeks, after which time no starting material was detected by TLC ($CHCl_3$). The reaction mixture was diluted with anhydrous ether, and the precipitate was filtered off under a stream of nitrogen, rinsed with anhydrous ether, and dried *in vacuo* to give yellow powder (2.40 g, 94%): ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52 (s, 2 H), 4.63 (q, 4 H), 1.50 (t, 6 H).

2,5-Bis(4-amidinophenyl)furan-d_8 dihydrochloride (1-d_8). A stirred suspension of **8** (0.50 g, 1.13 mmol) in anhydrous EtOH (35-40 mL) cooled in an ice-water bath was saturated with anhydrous ammonia and then sealed and allowed to warm to ambient temperature. Within a few hours, all solids went into solution, and the product began to

precipitate out soon thereafter. After 1 week, the reaction mixture was fitted with a condenser and refluxed for 1 hour. The cooled reaction mixture was neutralized with 1*N* NaOH, and a white solid was filtered off and rinsed with EtOH. The free base (0.33 g, 94%) was stirred for 5-6 hours in ethanolic HCl. The resulting dihydrochloride salt was filtered off and dried *in vacuo* at 60-70 °C for 3 days to give a yellow solid (0.38 g, 93%): mp 370-372 °C dec, (lit (1) 400-401 °C dec for unlabelled compound); ^{1}H NMR (400 MHz DMSO-d_6) δ9.46 (br s, 4 H), 9.21 (br s, 4 H), 8.10 (s, 0.030 H, residual), 7.96 (s, 0.032 H, residual) 7.; EIMS *m/z* (relative intensity) 312 (28.2, $C_{18}H_8D_8N_4O$); high resolution EIMS calcd for $C_{18}H_8D_8N_4O$ *m/z* 312.1826, found 312.1916. *Anal.* Calcd for $C_{18}H_8D_8N_4O$•2HCl (385.32): C, 56.10; H, 4.71; N, 14.54. Found: C, 56.24; H, 4.90; N, 14.23.

2,5-Bis-[4-(methoxyamidino)phenyl]furan-d_8 dihemimaleate (2-d_8). Methoxylamine hydrochloride (2.04 g, 24.4 mmol) was added to a solution of sodium ethoxide [prepared *in situ* from sodium (0.56 g, 24.4 mmol) and anhydrous EtOH (~75 mL)]. The suspension was stirred vigorously for 30 minutes, then added to **8** (1.35g, 3.04 mmol). The mixture was capped and stirred for 24 hours. The off-white suspension was then concentrated to half of its original volume and neutralized with 1*N* NaOH. A white solid was filtered off and dissolved in $CHCl_3$. The solution was dried (Na_2SO_4), and concentrated to near dryness to yield a suspension, which was diluted with ether. A pale yellow crystalline solid was filtered off as the free base (0.71 g, 63%): mp 209-210.5 °C; IR *v* 3495, 3456,3392, 3317, 1628, 1365, 1052, 905 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ7.15 (s, 2 H), 6.06 (br s, 4 H), 3.76 (s, 6H).

A mixture of the above diamidoxime base (0.37 g, 1.0 mmol) and maleic acid (0.24 g, 2.0 mmol) in dry MeOH (30 mL) was stirred at RT. The resulting solution was then filtered, and and the filtrate was concentrated *in vacuo* to give an oil which solidified under vacuum. The yellow solid was triturated with ether, then filtered off and dried under vacuum at 50-60 °C for 3 days to give the title compound as a pale yellow/tan solid (0.49 g, 81%): mp 140-142 °C (dec); ^{1}H NMR (400, MHz, DMSO-d_6 + D_2O) δ7.84 (s, 0.024 H, residual), 7.74 (s, 0.024 H, residual), 7.15 (s, 2 H), 6.24 (s, 4 H), 3.77 (s, 6 H). *Anal.* Calcd for $C_{28}H_{20}D_8N_4O_{11}$ (604.60): C, 55.62; H + D as H, 4.67; N, 9.27. Found: C, 55.26; H + D as H, 4.81; N, 9.37.

4,4'-Dibromobiphenyl-d_8 (9). Pulverized biphenyl-d_{10} (6.57 g, 40.0 mmol) was placed in an evaporating dish on the rack of a vacuum desiccator. Bromine (5.6 mL, 109 mmol) was placed in another dish beneath the rack. The biphenyl was left in contact with the bromine vapor for 16 h, with the desiccator vented in a fume hood. The excess bromine and hydrogen bromide were allowed to evaporate from the crude product (ca. 12 g, a mixture of mono-dibromination products) upon standing for several hours. The crude product was recrystallized from toluene/hexane to give white crystals (8.23 g) containing the monobromo impurity (6 area % by HPLC.) (The monobromide recovered from the mother liquors can be brominated in acetic acid/carbon tetrachloride to give more of the desired product). A second recrystallization gave white crystals (4.98 g, 38.9%): mp 163-165 °C (lit (13) 162-163 °C for unlabelled compound), CIMS m/z (relative intensity) 322 (54, $C_{12}D_8{}^{81}Br_2$), 320 (100, $C_{12}D_8{}^{79}Br^{81}Br$), 318 (52, $C_{12}D_8{}^{79}Br_2$); HPLC (method B) t_R 9.46 min (99.0 area %). *Anal.* Calcd for $C_{12}D_8Br_2$ (320.06): C, 45.03; D as H, 2.52; Br, 49.93. Found: C, 45.27; H, 2.54; Br, 49.67

4,4'-Dicyanobiphenyl-d_8 (10). Dried copper (I) cyanide (4.50 g, 50.2 mmol) was added to a solution of 4,4'-dibromobiphenyl-d_8 (**9**, 6.01 g, 18.8 mmol) in anhydrous DMF (50 mL). The mixture was stirred at reflux under nitrogen for 10 hours. The cooled reaction mixture was poured over ice. The resulting precipitate was filtered off and stirred in aqueous ethylenediamine under gentle heat. The suspension was poured in ethyl acetate (400 mL), and the biphasic mixture was stirred until all solids were dissolved. Layers were separated in a separatory funnel. The aqueous layer was extracted with ethyl acetate (2 x 150 mL). The combined extracts were washed successively with 10% NaCN solution, water, and brine. The extract was evaporated to a white powder (3.89 g, 97.6%): mp 335-337 °C; HPLC (method B) t_R 2.79 min (95.1 area %). *Anal.* Calcd for $C_{14}D_8N_2 \cdot 0.1H_20$ (214.08): C, 78.55; H + D as H, 3.86; N, 13.09. Found: C, 78.71; H + D as H, 3.88; N, 12.94.

4,4'-Dicyano-2-nitrobiphenyl-d_7 (11). A solution of nitric acid (70%, 2.05 g, 22.6 mmol) in sulfuric acid (15 mL) was added dropwise to a solution of 4,4'-dicyanobiphenyl-d_8 (**10**, 3.89 g, 18.3 mmol) in sulfuric acid (60 mL) at such a rate that the temperature of the reaction mixture was maintained below 0 °C. The mixture was

stirred for 1 h, after which time no starting material was detected by HPLC. The reaction mixture was poured over ice water (500 mL). The aqueous suspension was extracted twice with ethyl acetate. The combined organic layers were washed 3 times with saturated $NaHCO_3$ solution, until the aqueous layer was colorless and neutral in pH. The extracts were then washed successively with water and brine, then dried ($MgSO_4$) and evaporated. The crude product was purified by flash chromatography on silica gel 200-425 mesh, 5 cm (o.d.) x 23 cm bed volume, eluting with 35% ethyl acetate in hexane, to give a pale yellow powder (2.21 g, 47.1%): mp 210-217 °C; HPLC (method B) t_R 2.42 min (100 area %). *Anal.* Calcd for $C_{14}D_7N_3O_2$ (256.27): C, 65.61, D as H, 2.75; N, 16.40. Found: C, 65.38; D as H, 2.82; N, 16.24.

2,7-Dicyanocarbazole-d_6 (12). Triisopropyl phosphite (6.5 mL, 26 mmol) was added to a solution of 4,4'-dicyano-2-nitrobiphenyl-d_7 (**11**, 2.21g, 8.62 mmol) in refluxing toluene (75 mL). After 72 h the excess reagent was distilled off. The crude product was purified by flash chromatography on silica gel 200-425 mesh, 5 cm (o.d.) x 18 cm bed volume, eluting with 20 to 40% ethyl acetate in hexane to give a yellow powder: 0.81 g (42%); HPLC (method B) t_R 2.19 min (96.3 area %), (method A) 10.07 min (98.2 area %), identical to authentic unlabelled compound by TLC and HPLC.

2,7-Diamidinocarbazole-d_6 *hydrochloride* (3-d_6). 2,7-Dicyanocarbazole-d_6 (**12**, 0.78 g, 3.5 mmol) was added to a solution of anhydrous ethanol (10 mL, 170 mmol) in 1,4-dioxane (50 mL) which had been saturated with hydrogen chloride and cooled to < 0 °C. The sealed reaction mixture was allowed to warm to room temperature and stirred for 6 days. The reaction mixture was diluted with ether, and the crude diimidate (1.14 g, 84%) was filtered off under nitrogen and dried *in vacuo*. A suspension of the crude diimidate in anhydrous ethanol (50 mL) was cooled to 0 °C and saturated with ammonia. The sealed reaction mixture was stirred at room temperature overnight. It was then heated at 40-50° for 8 hours, and stirring continued overnight at room temperature. The precipitate was filtered off. The solid was suspended in hot ethanol (15 mL). The suspension was allowed to cool, then stored briefly at –20 °C before the solid was filtered off to give an ivory powder (0.50 g, 47%): mp > 300 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ8.70 (br s); HPLC (method A) t_R 2.85 min (95.8 area %). *Anal.* Calcd for

$C_{14}H_7D_6N_5 \cdot HCl \cdot 0.2C_2H_5OH$: C, 57.08; H + D as H, 5.06; N, 23.11; Cl, 11.70. Found: C, 56.73; H + D as H, 4.97; N, 22.83; Cl, 11.96.

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References

(1) Das, B. P.; Boykin, D. W. *J. Med. Chem.* **20**: 531-536 (1977).

(2) Boykin, D. W.; Kumar, A.; Spychala, J.; Zhou, M.; Lombardy, R. J.; Wilson, W. D.; Dykstra, C. C.; Jones, S. K.; Hall, J. E.; Tidwell, R. R.; Laughton, C.; Nunn, C. M.; Neidle, S. *J. Med. Chem.* **38**: 212-916 (1995).

(3) Patrick, D. A.; Boykin, D. W.; Wilson, W. D.; Tanious, F. A.; Spychala, J.; Bender, B. C.; Hall, J. E.; Dykstra, C. C.; Ohemeng, K. A.; Tidwell, R. R. *Eur. J. Med. Chem.* **32**: 781-793 (1997).

(4) Hall, J. E.; Kerrigan, J. E.; Ramachandran, K.; Bender, B. C.; Stanko, J. P.; Jones, S. K.; Patrick, D. A.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **42**: 666-674 (1998).

(5) Blagburn, B. L.; Drain, K. L.; Land, T. M.; Moore, P. H.; Kinard, R. G.; Lindsay, D. S.; Kumar, A.; Shi, J.; Boykin, D. W.; Tidwell, R. R. *J. Parasitol.* **84**: 851-856 (1998).

(6) Blagburn, B. L.; Drain, K. L.; Land, T. M.; Kinard, R. G.; Moore, P. H.; Lindsay, D. S.; Patrick, D. A.; Boykin, D. W.; Tidwell, R. R. *Antimicrob. Agents Chemother.* **42**: 2877-2882 (1998).

(7) Boykin, D. W.; Kumar, A.; Hall, J. E.; Bender, B. C.; Tidwell, R. R. *Bioorg. Med. Chem. Lett.* **6**: 3017-3020 (1996).

(8) Stetter, H.; Kuhlmann, H. *Org. React.* **40**: 407-493 (1991).

(9) Bajic, M.; Kumar, A.; Boykin, D. W. *Heterocyclic Comm.* **2**: 135-140 (1996).

(10) Kumar, A.; Stephens, C. E.; Boykin, D. W. *Heterocyclic Comm.* **5**: 301-304 (1999).

(11) Lutz, R. E.; McGinn, C. E. *J. Am. Chem. Soc.* **64**: 2585-2588 (1942).

(12) Yamato, T.; Hideshima, C.; Suehiro, K.; Tashiro, M.; Prakash, G. K. S.; Olah, G. A. *J. Org. Chem.* **56**: 6248-6250 (1991).

(13) Buckles, R. E.; Wheeler, N. G. *Org. Synth.* **Coll. IV**: 256-258 (1963).

(14) Cadogan, J. I. G.; Cameron-Wood, M.; Mackie, R. K.; Searle, R. J. G. *J. Chem. Soc.* 4831-4837 (1965).

(15) Conant, J. B.; Lutz, R. E. *J. Am. Chem. Soc.* **47**: 881-892 (1925).

(16) Hale, W. J.; Thorp, L. *J. Am. Chem. Soc.* **35**: 262-272 (1913).

(17) Hiskey, R. G.; Thomas, B. D.; Kepler, J. A. *J. Org. Chem.* **29**: 3671-3677 (1964).

(18) Lutz, R. E.; Rowlett, R. J., Jr. *J. Am. Chem. Soc.* **70**: 1359-1363 (1948).

