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Research Article

Synthesis of deuterium and ^{15}N-labelled 2,5-*Bis*[5-amidino-2-pyridyl]furan and 2,5-*Bis*[5-(methoxyamidino)-2-pyridyl]furan

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Summary

The acetate salt of 2,5-*bis*[5-amidino-2-pyridyl]furan-d_2/$^{15}N_2$ (**4**) was synthesized from 2,5-*bis*[5-cyano-2-pyridyl]furan-d_2 (**2**), through the *bis*-*O*-acetoxyamidoxime followed by hydrogenation. Compound **2** was obtained via a Stille coupling reaction of 6-chloronicotinonitrile with 2,5-*bis*[tri-*n*-butyltin]-furan-d_2 (**1**). 2,5-*bis*[5-amidino-2-pyridyl)furan-d_6 (**10**) was synthesized from 2,5-*bis*[5-cyano-2-pyridyl)furan-d_6 (**9**) *via* a direct reaction with lithium *bis*(trimethylsilyl)amide, followed by deprotection with ethanolic HCl. ^{15}N and/or deuterium-labelled methoxy-amidines **5a**-d_2/$^{15}N_2$, **5b**-d_8, **12**, **14**-d_6 were prepared in good yield *via* direct methylation of their respective diamidoximes with either dimethylsulfate-d_0 or dimethylsulfate-d_6 in DMF solution and using LiOH as a base. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: deuterium-labelled; ^{15}N-isotopes; prodrug; bromination; Stille coupling

Introduction

2,5-*Bis*[4-methoxyamidinophenyl]furan (**Ia**), an orally administered prodrug, is currently in Phase II clinical trials against malaria, and Phase III trials against *Pneumocystsis pneumonia* and human African trypanosomiasis (HAT).[1–7] The prodrug undergoes a multistep bioconversion *in vivo* to yield the active drug furamidine (**IIa**).[8–11] The establishment of the bioconversion

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pathway of **Ia** into **IIa** was significantly aided by the synthesis of deuterium labelled **Ia** and **IIa**.[12]

As part of an effort to develop antitrypanosomal compounds that are more effective than **Ia/IIa** we have found that aza-analogs of these compounds show excellent activity against *Trypanosoma brucei rhodesiense* (*T. b. r.*) both *in vitro* and *in vivo* in a mouse model.[13] Specifically, **Ib** which is a prodrug of **IIb**, has shown very promising oral activity giving cures in the virulent STIB900 mouse model *T. b. r.* at the low dosage of 5 mg/kg which is superior to the activity of **Ia** in the same model. The establishment of the bioconversion pathway of **Ib** into **IIb** was significantly aided by the synthesis of deuterium labelled **Ib** and **IIb**.[14,15] In view of the efficacy of **III** against *T. b. r.* and its putative simpler metabolism than **Ib**, preclinical toxicity and metabolism studies have been initiated in anticipation of further trials. It is expected that labelling of the furan group of **III** and **IV** with deuterium (d_2-analog) plus two nitrogen atoms with the ^{15}N-isotope will serve as reasonable mass spectroscopy internal standards for metabolism studies. For comparative metabolism studies we also require the d_6 and d_8 analog of **III**. It is hypothesized that metabolism of **III** will likely be similar to that of **Ia**, because **III** is a symmetric molecule as in **Ia**. Consequently, to aid with these studies we describe the syntheses of the novel isotopically labelled compounds **3a-d_2/$^{15}N_2$**, **4-d_2/$^{15}N_2$**, **5a-d_2/$^{15}N_2$**, **5b-d_8**, **10**, **11**, **12** and **14-d_6** for use in preclinical studies (Figure 1).

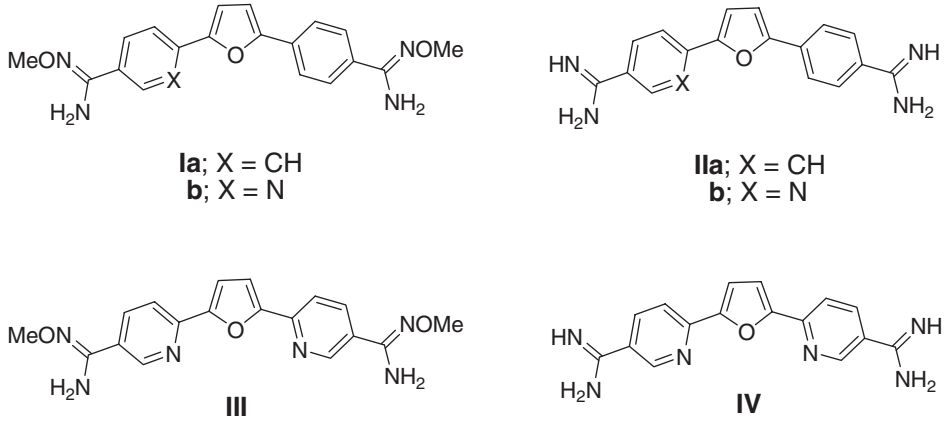

Figure 1.

Results and discussion

As shown in Scheme 1, the acetate salt of 2,5-*bis*[5-amidino-2-pyridyl]furan-d_2/$^{15}N_2$ (**4**) was synthesized from 2,5-*bis*[5-cyano-2-pyridyl]furan-d_2 (**2**), through the *bis*-*O*-acetoxyamidoxime followed by hydrogenation. Compound **2** was obtained *via* a Stille coupling reaction of 6-chloronicotinonitrile with

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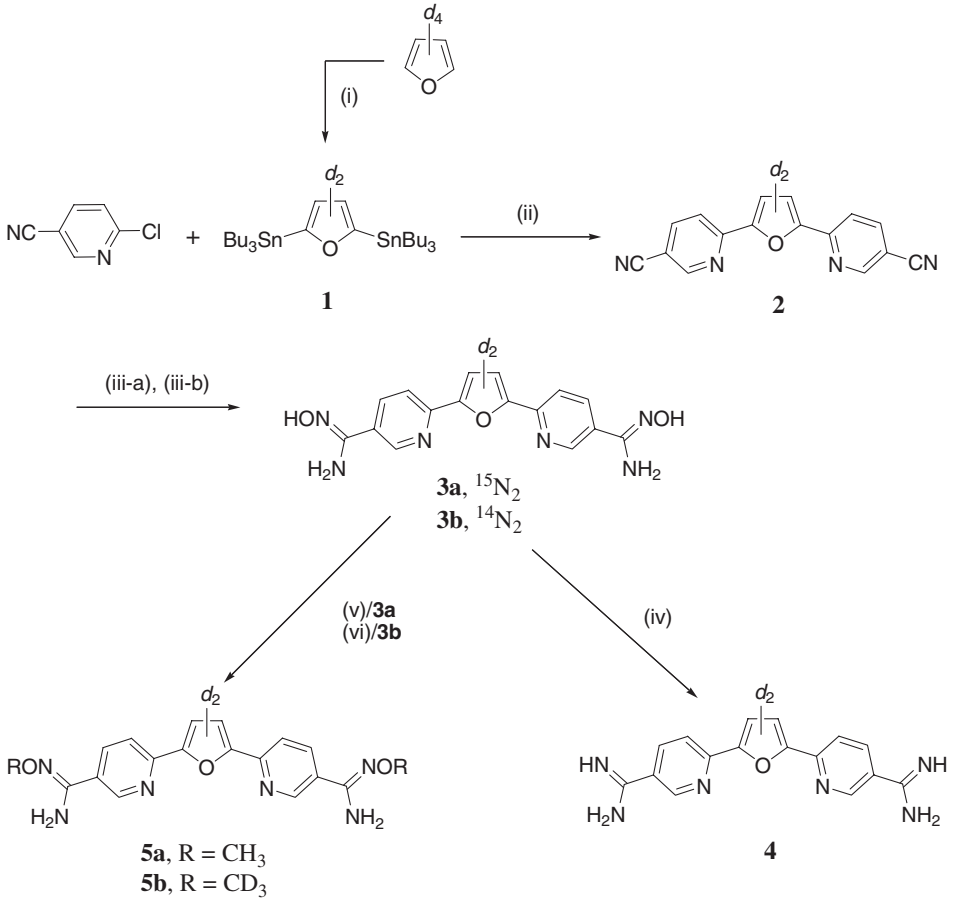

Scheme 1. Reagents and conditions: (i) *sec*.BuLi, Bu_3SnCl; (ii) $Pd(PPh_3)_4$ 1,4-dioxane; (iii-a) $^{15}NH_2OH.HCl/KO$-*t*-Bu, (iii-b) $NH_2OH.HCl/KO$-*t*-Bu; (iv-a) $AcOH/Ac_2O$, (iv-b) H_2/Pd-C; (v) $(CH_3)_2SO_4/LiOH$; (vi) $(CD_3)_2SO_4/LiOH$

2,5-*bis*[tri-*n*-butyltin]-furan-d_2 (**1**), which was prepared from commercially available furan-d_4 v*ia* lithiation with *sec*-butyllithium and a subsequent treatment with tri-*n*-butyltin chloride as previously described for furan-d_0.[16] 2,5-*Bis*[5-(methoxyamidino)-2-pyridyl]furan-$d_2/^{15}N_2$ (**5a**) was prepared in good yield via methylation of the diamidoxime **3a**-$d_2/^{15}N_2$, a product of the treatment of the dinitrile **2** with ^{15}N-labelled hydroxylamine hydrochloride and potassium *tert*-butoxide in DMSO at ambient temperature, with dimethylsulfate in DMF solution and using LiOH as a base. In a similar way, 2,5-*bis* [5-(methoxyamidino)-2-pyridyl]furan-d_8 (**5b**) was prepared via methylation of the diamidoxime **3b**-d_2 with dimethylsulfate-d_6.

As outlined in Scheme 2, 2,5-*bis*[5-amidino-2-pyridyl]furan-d_6 (**10**) was synthesized from 2,5-*bis*[5-cyano-2-pyridyl]furan-d_6 (**9**). The dinitrile **9** was

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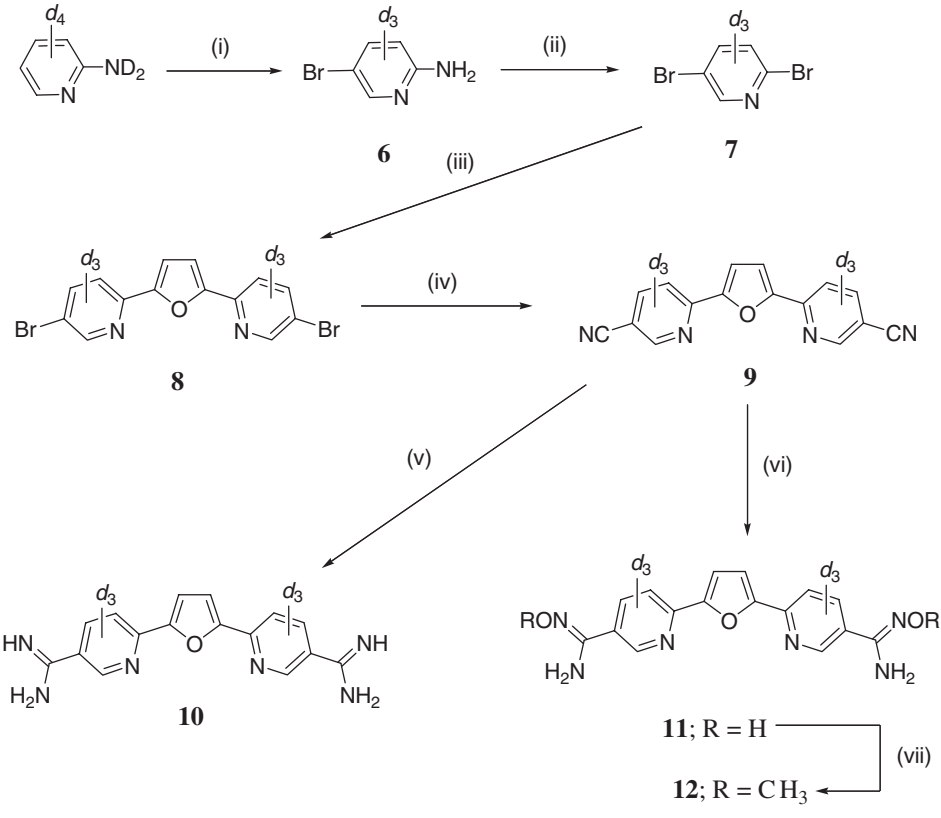

Scheme 2. Reagents and conditions: (i) NBS, DMF; (ii) aq. HBr, Br_2, $NaNO_2$, 0°C to Rt; (iii) 2,5-*bis*[tri-*n*-butyltin]-furan, $Pd(PPh_3)_4$; (iv) Cu(I)CN, DMF; (v) a) $LiN(TMS)_2$, THF, (v-b) HCI(g)/EtOH; (vi) $NH_2OH \cdot HCl$/KO-*t*-Bu; (vii) $(CH_3)_2SO_4$/LiOH

allowed to react with lithium *bis*(trimethylsilyl)amide[17] in THF, followed by deprotection of the silylated amidines with ethanolic HCl to furnish a hydrochloride salt of the diamidine **10**. Compound **9** was obtained in four steps starting with bromination of the commercially available 2-aminopyridine-d_6 with *N*-bromosuccinimide in DMF solution to give 5-bromo-2-aminopyridine-d_3 (**6**). Treatment of **6** with hydrobromic acid, bromine and sodium nitrite,[18] gave 2,5-dibromopyridine-d_3 (**7**) in 76% yield. A Stille coupling reaction of two equivalents of **7** with one equivalent of 2,5-*bis*[tri-*n*-butyltin]-furan-d_0 at 70–80°C furnished the dibromo-compound **8**, the regioselectivity of **7** is consistent with similar reactions reported previously for 2,5-dibromopyridine-d_0.[13,19–22] Cyanation of **8** with Cu(I)CN at 140–150°C gave the dinitrile **9**. Treatment of **9** with hydroxylamine hydrochloride and potassium *tert*-butoxide in DMSO at ambient temperature gave the diamidoxime **11** in 93% yield. A subsequent methylation of

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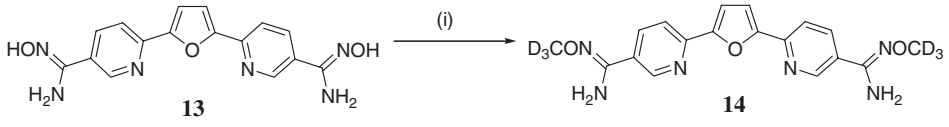
11 with dimethylsulfate-d_0 afforded 2,5-*bis*[5-(methoxyamidino)-2-pyridyl] furan-d_6 (**12**).

2,5-*Bis*[5-(methoxyamidino)-2-pyridyl]furan-d_6 (**14**) which bears deuterium-labelled methoxy groups was prepared by direct methylation of the respective diamidoxime (**13**)[13] with dimethylsulfate-d_6 (Scheme 3). The hydrochloride salts of all the oximes, **3a**-$d_2/^{15}N_2$, **5a**-$d_2/^{15}N_2$, **5b**-d_8, **11**, **12**, **14**-d_6 were made by passing hydrogen chloride gas into ethanolic solutions of their free bases.



Scheme 3. Reagents and conditions: (i) $(CD_3)_2SO_4$/LiOH

Conclusion

An efficient five step synthesis of **4**-$d_2/^{15}N_2$ (**IV**-isotope) and four step syntheses of **5a**-$d_2/^{15}N_2$ and **5b**-d_8 (**III**-isotopes) starting from furan-d_4 have been developed. In addition, labelling of **III** has been achieved in two more different ways, a six-step approach for the synthesis of **12**-d_6 starting from 2-aminopyridine-d_6 has been described and direct methylation of **13** with dimethylsulfate-d_6 provided **14**-d_6. No detectable exchange of deuterium was observed during the syntheses of all of the target compounds. The use of these deuterium labelled compounds in metabolism and pharmacokinetic studies will be described in due course.

Experimental section

Melting points were recorded using a Thomas-Hoover (Uni-Melt) capillary melting point apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F_{254} precoated aluminum sheets and detected under UV light. ^{1}H and ^{3}C NMR spectra, were recorded employing a Varian Unity Plus 300 spectrometer (Varian, Inc., Palo Alto, California, USA), and chemical shifts (δ) are in ppm relative to TMS as internal standard. Mass spectra were recorded on a VG analytical 70-SE spectrometer (VG Analytical, Ltd., Manchester, UK). Elemental analyses were obtained from Atlantic Microlab Inc. (Norcross, GA) and are within $\pm$ 0.4 of the theoretical values. The compounds reported as salts frequently analyzed correctly for fractional moles of water and/or ethanol of solvation. In each case proton NMR showed the presence of indicated solvent(s). All non-labelled chemicals and solvents were purchased from Aldrich Chemical Co. or Fisher Scientific. Isotopically labelled compounds, furan-d_4 (98 atom % D), hydroxylamine.HCl (98 atom % ^{15}N), 2-aminopyridine-d_6 (98 atom % D) and dimethylsulfate-d_6 (99 atom % D) were obtained from Aldrich Chemical Co., Cambridge Isotopes or Icon

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Isotopes. NBS was recrystallized from nitromethane prior to use. All solvents were reagent grade.

2,5-Bis(tri-n-butylstannyl)furan-d_2 (**1**)

To a solution of furan-d_4 (2.2 ml, 30 mmol), tetramethylethylenediamine (11.3 ml, 75 mmol), and hexane (75 ml) under nitrogen was added *sec*.butyllithium (53.5 ml, 75 mmol) dropwise at 0°C. After 1 h at 0°C, the reaction mixture was warmed to room temperature and kept stirring for 8 h, thereafter the reaction mixture was cooled to 0°C and a solution of *n*-tributyltin chloride (21.8 ml, 81 mmol) was added dropwise. The reaction mixture was warmed to room temperature and kept stirring for 48 h, then 75 ml of saturated ammonium chloride was added and the product was extracted using hexanes. The organic layer was washed with aqueous $CuSO_4$ (2.5%), dried (Na_2SO_4) and the solvents removed under reduced pressure to give **1** as an oil in 85% yield. ^{1}H NMR ($CDCl_3$); δ 1.58 (m, 12 H), 1.36 (m, 12 H), 1.06 (t, J = 8.1 Hz, 12 H), 0.91 (m, 18 H). EIMS (m/z, relative intensity (rel.int.)); 647 ($M^+ + 1$, 20), 646 (M^+, 15), 591 (75), 535 (30), 481 (15), 421 (20), 367 (20), 177 (90), 57 (1 0 0).

2,5-Bis[5-cyano-2-pyridyl)furan-d_2 (**2**)

A mixture of 6-chloronicotinonitrile (4.14 g, 30 mmol), 2,5-*bis*(tri-*n*-butylstannyl)furan-d_2 (9.93 g, 15 mmol) and tetrakis(triphenyl-phosphine)palladium(0) (900 mg) in dry 1,4-dioxane (40 ml) was heated under nitrogen at 80–90°C for 24 h. The solvent was evaporated under reduced pressure and the residue was dissolved in methylene chloride and the solution was passed through celite to remove Pd. The solution was evaporated, the precipitate was triturated with ethanol, filtered, washed with hexanes to furnish **2** in 75% yield, m.p. 308–310°C (DMF), identical to authentic unlabelled compound by TLC (R_f = 0.48 using hexanes/ethyl acetate, 60:40). ^{1}H NMR (DMSO-d_6); δ 8.14 (d, J = 8.4 Hz, 2 H), 8.38 (dd, J = 8.4, 1.8 Hz, 2 H), 9.02 (d, J = 1.8 Hz, 2 H). ^{13}C NMR (DMSO-d_6); 153.3, 152.5, 149.9, 140.7, 118.6, 116.7, 107.1. MS (ESI) m/e (rel. int.): 275 ($M^+ + 1$, 100). High resolution mass calculated for $C_{16}H_7D_2N_4O$: 275.0902. Observed 275.0890. *Analytically* Calculated for $C_{16}H_6D_2N_4O$-0.1H_2O: C, 69.60; H + D as H, 3.02. Found. C, 69.49; H + D as H, 2.97.

2,5-Bis[5-(N-hydroxyamidino)-2-pyridyl]furan-d_2/$^{15}N_2$ (**3a**)

A mixture of hydroxylamine hydrochloride, ^{15}N-isotope, (2.11 g, 30 mmol, 10 eq.) in anhydrous DMSO (25 ml) was cooled to 5°C under nitrogen and potassium *t*-butoxide (3.36 g, 30 mmol, 10 eq.) was added in portions. The mixture was stirred for 30 min. To this mixture was added the dinitrile-d_2 **2**

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(822 mg, 3 mmol, 1 eq.). The reaction mixture was stirred for 24 h at room temperature. The reaction mixture was then poured slowly onto ice–water. The precipitate was filtered and washed with water and then ethanol to afford **3a** (free base) in 98% yield; m.p. 265–267°C. ^{1}H NMR (DMSO-d_6); δ 6.30 (s, 4H), 7.96 (d, J = 8.4 Hz, 2H), 8.15 (dd, J = 8.4, 2.4 Hz, 2H), 8.91 (d, J = 2.4 Hz, 2H), 10.00 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 153.5, 149.9, 148.4, 147.1, 134.2, 126.6, 118.2. MS (ESI) m/e (rel. int.): 343 ($M^+ + 1$, 100), 225 (10). High resolution mass calculated for $C_{16}H_{13}D_2^{14}N_4^{15}N_2O_3$: 343.1272. Observed 343.1259. (**3a hydrochloride salt**); m.p. 291–293.5°C dec. *Analytically* Calculated for $C_{16}H_{12}D_2^{14}N_4^{15}N_2O_3$-4.0 HCl: C, 39.33; H + D as H, 3.73; N, 17.21. Found. C, 39.49; H + D as H, 3.91; N, 17.29.

*2,5-Bis[5-(N-hydroxyamidino)-2-pyridyl]furan-d_2 (**3b**)*

The same procedure described for **3a** was used by employing $^{14}NH_2OH.HCl$ instead of $^{15}NH_2OH.HCl$. Yield 95%; m.p. 286–288°C. ^{1}H NMR (DMSO-d_6); δ 5.89 (s, 4H), 7.93 (d, J = 8.1 Hz, 2H), 8.12 (dd, J = 8.1, 2.4 Hz, 2H), 8.90 (d, J = 2.4 Hz, 2H), 9.78 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 153.5, 148.7, 148.0, 146.8, 133.7, 127.6, 118.1. MS (ESI) m/e (rel. int.): 341 ($M^+ + 1$, 100), 324 (20), 307 (25), 241 (10), 214 (10), 171 (80). High resolution mass calculated for $C_{16}H_{13}D_2N_6O_3$: 341.1331. Observed 341.1330.

*2,5-Bis[5-amidino-2-pyridyl)furan-d_2/$^{15}N_2$ acetate salt (**4**)*

To a solution of **3a** (342 mg, 1 mmol) in glacial acetic acid (10 ml) was slowly added acetic anhydride (0.35 ml). After stirring overnight TLC indicated complete acylation of the starting material. The reaction mixture was poured onto ice-water, the precipitate was filtered, washed with water and dried. To the precipitate in glacial acetic acid (13 ml), and ethanol (20 ml) was added 10% palladium on carbon (150 mg). The mixture was placed in a Parr hydrogenation apparatus at 50 psi for 8 h at room temperature. The mixture was filtered through hyflo and the filter pad washed with water. The filtrate was evaporated under reduced pressure and the precipitate was collected and washed with ether to give **4** in 73% yield, m.p. 242–244°C dec. ^{1}H NMR (D_2O/DMSO-d_6); δ 1.78 (s, 3.5 × CH_3 of acetate) 8.06 (d, J = 8.1 Hz, 2H), 8.25 (dd, J = 8.1, 1.8 Hz, 2H), 8.96 (d, J = 1.8 Hz, 2H). MS (ESI) m/e (rel. int.): 311 ($M^+ + 1$, 40), 294 (10), 156 (100). High resolution mass calculated for $C_{16}H_{13}D_2^{14}N_4^{15}N_2O$: 311.1374. Observed 311.1371. *Analytically* Calculated for $C_{16}H_{12}D_2^{14}N_4^{15}N_2O-3.5CH_3CO_2H-1.75H_2O$: C, 49.99; H + D as H, 5.70; N, 15.22. Found. C, 49.80; H + D as H, 5.73; N, 15.46.

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2,5-Bis[5-(methoxyamidino)-2-pyridyl]furan-d_2/$^{15}N_2$ (**5a**)

To a suspension of the diamidoxime **3a** (376 mg, 1.1 mmol) in DMF (15 ml) was added $LiOH.H_2O$ (554 mg, 13.2 mmol, in 5 ml H_2O) which was followed by dimethylsulfate (1.38 g, 11 mmol). The reaction mixture was kept stirring overnight after which it was poured onto ice/water and the precipitate was filtered, washed with water and dried to give the desired compound in 66% yield; m.p. 233–234.5°C. ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 6H), 6.31 (s, 4H), 7.97 (d, J = 8.4 Hz, 2H), 8.12 (dd, J = 8.4, 2.1 Hz, 2H), 8.87 (d, J = 2.1 Hz, 2H). ^{13}C NMR (DMSO-d_6); δ 153.5, 148.9, 148.3, 146.9, 134.1, 126.8, 118.0, 60.7. MS (ESI) m/e (rel. int.): 371 ($M^+ + 1$, 100), 343 (10), 253 (10). High resolution mass calculated for $C_{18}H_{17}D_2^{14}N_4^{15}N_2O_3$: 371.1585. Observed 371.1588. (**5a hydrochloride salt**); m.p. 204–206°C dec. *Analytical* Calculated for $C_{18}H_{16}D_2^{14}N_4^{15}N_2O_3$-4.0HCl-1.8$H_2O$: C, 39.37; H + D as H, 4.66; N, 15.31. Found. C, 39.25; H + D as H, 4.62; N, 15.21.

2,5-Bis[5-(methoxyamidino)-2-pyridyl]furan-d_8 (**5b**)

The target compound was prepared adopting the same procedure described for **5a**, starting with diamidoxime **3b** and employing dimethylsulfate-d_6 instead of dimethylsulfate-d_0. Yield 64%, m.p. 234–236°C. ^{1}H NMR (DMSO-d_6): δ 6.29 (s, 4H), 7.96 (d, J = 8.4 Hz, 2H), 8.11 (dd, J = 8.4, 2.1 Hz, 2H), 8.87 (d, J = 2.1 Hz, 2H). ^{13}C NMR (DMSO-d_6); δ 153.5, 148.9, 148.3, 147.0, 134.2, 126.9, 118.1. MS (ESI) m/e (rel. int.): 375 ($M^+ + 1$, 100), 188 (35). High resolution mass calculated for $C_{18}H_{11}D_8N_6O_3$: 375.2021. Observed 375.2015. (**5b hydrochloride salt**); m.p. 198–200°C dec. *Analytical* Calculated for $C_{18}H_{10}D_8N_6O_3$-4.0HCl-1.5H_2O: C, 39.50; H + D as H, 4.58; N, 15.35. Found. C, 39.27; H + D as H, 4.72; N, 15.13.

5-Bromo-2-aminopyridine-d_3 (**6**)

To a solution of 2-aminopyridine-d_6 (5.00 g, 50 mmol) in DMF (20 ml) was added portionwise *N*-bromosuccinimide (8.90 g, 50 mmol) with stirring. The reaction mixture was stirred overnight at room temperature, then poured onto cold-water. The precipitate was collected, washed with water and dried then purified using chromatography (SiO_2, hexanes/ethyl acetate, 40: 60) to furnish **6** in 81% yield, m.p. 132–133°C, identical to authentic unlabelled compound by TLC (R_f = 0.60 using hexanes/ethyl acetate, 40:60). ^{1}H NMR (DMSO-d_6): δ 6.12 (s, 2H). MS (ESI) m/e (rel. int.): 176, 178 ($M^+ + 1$, 100, 90). High resolution mass calculated for $C_5H_3D_3BrN_2$: 175.9903. Observed 175.9902.

2,5-dibromopyridine-d_3 (**7**)

To a solution of **6** (5.46 g, 31 mmol) in 48% aqueous hydrobromic acid (48 ml) was added 5.0 ml of bromine at 0°C and 24.2 ml of an aqueous solution of

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$NaNO_2$ (3.25 M). The reaction mixture was stirred at room temperature for 1 h and then neutralized with NaOH (1N, 435 ml). The unreacted bromine was quenched with $Na_2S_2O_3$, the product was extracted with ethyl acetate. The organic layers were combined, washed with water, dried over anhydrous Na_2SO_4 and concentrated to dryness. The residue was chromatographed on silica gel using hexanes/ethyl acetate (95 : 5) as an eluent to give **7** in 76% yield, m.p. 94.5–95.5 °C, identical to authentic unlabelled compound by TLC (R_f = 0.81 hexanes/ethyl acetate, 95:5). MS (ESI) *m/e* (rel. int.): 239, 241 ($M^+ + 1$, 40, 100), 175 (10). High resolution mass calculated for $C_5HD_3Br_2N$: 238.8899. Observed 238.8894. *Analytical* Calculated for $C_5D_3Br_2N$: C, 25.03; H + D as H, 1.25. Found. C, 25.17; H + D as H, 1.27.

*2,5-Bis[5-bromo-2-pyridyl)furan-d_6 (**8**)*

2,5-dibromopyridine-d_3 (**7**) (4.78 g, 20 mmol, 2 eq.) and 2,5-*bis*(tri-*n*-butyl-stannyl)furan-d_0 (6.60 g, 10 mmol, 1 eq.) were reacted under the above mentioned Stille coupling conditions to give the target compound **8** in 61% yield, m.p. 211–213°C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.28 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 152.8, 146.4, 118.1, 111.6. MS (ESI) *m/e* (rel. int.): 385, 387 ($M^+ + 1$, 40, 100), 317 (35). High resolution mass calculated for $C_{14}H_3D_6Br_2N_2O$: 384.9458. Observed 384.9457. *Analytical* Calculated for $C_{14}H_2D_6Br_2N_2O$: C, 43.55; H + D as H, 2.10. Found. C, 43.63; H + D as H, 2.09.

*2,5-Bis[5-cyano-2-pyridyl)furan-d_6 (**9**)*

A mixture of 2,5-*bis*[5-bromo-2-pyridyl)furan-d_6 (**8**) (2.20 g, 5.75 mmol) and Cu(1)CN (2.04 g, 23 mmol) in DMF (40 ml) was heated at 140–150°C for 24 h. The reaction mixture was poured onto water and the solid which formed was extracted with methylene chloride (300 ml, 3 ×) from aq. NH_4OH. The solvent was evaporated and the precipitate was purified by chromatography (SiO_2, ethyl acetate/hexanes 90:10). Yield 55%, m.p. 304–306°C, identical to authentic unlabelled compound by TLC. ^{1}H NMR (DMSO-d_6): δ 7.50 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 153.3, 149.9, 116.6, 114.4, 106.9. MS (ESI) *m/e* (rel. int.): 279 ($M^+ + 1$, 100), 254 (70). High resolution mass calculated for $C_{16}H_3D_6N_4O$: 279.1146. Observed 279.1017. *Analytical* Calculated for $C_{16}H_2D_6N_4O$-0.25H_2O: C, 67.93; H + D as H, 3.03. Found. C, 67.66; H + D as H, 3.20.

*2,5-Bis[5-amidino-2-pyridyl)furan-d_6 (**10**)*

The dinitrile-d_6 **9** (278 mg, 1 mmol), suspended in freshly distilled THF (5 ml), was treated with lithium trimethylsilylamide (1 M solution in THF, 4 ml, 4 mmol) and the reaction was allowed to stir overnight. The reaction mixture

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was then cooled to 0°C to which was added HCl saturated ethanol (70 ml) whereupon a precipitate started forming. The mixture was left to run overnight whereafter it was diluted with ether and the resultant solid was collected by filtration. The diamidine was purified by neutralization with 1N NaOH followed by filtration of the resultant solid and washing with water (3X). Finally, the free base was stirred with ethanolic HCl overnight, diluted with ether, and the solid formed was filtered and dried to give the diamidine salt **10** in 90% yield; m.p. >300°C. ^{1}H NMR (DMSO-d_6): δ 7.54 (s, 2H), 9.44 (s, 4H), 9.70 (s, 4H). ^{13}C NMR (DMSO-d_6); δ 163.4, 153.7, 151.2, 122.6, 114.4. MS (ESI) *m/e* (rel. int.): 313 ($M^+ + 1$, 70), 230 (25), 157 (1 0 0). High resolution mass calculated for $C_{16}H_9D_6N_6O$: 313.1684. Observed 313.1689. (**10** hydrochloride salt) *Analytical* Calculated for $C_{16}H_8D_6N_6O$-4.0HCl-1.0H_2O-0.6C_2H_5OH: C, 41.00; H + D as H, 4.68; N, 16.67. Found. C, 41.22; H + D as H, 4.58; N, 16.32.

*2,5-Bis[5-(N-hydroxyamidino)-2-pyridyl]furan-d_6 (**11**)*

The same procedure described for **3a** was used, starting with dinitrile-d_6 **9** and employing $^{14}NH_2OH$.HCl instead of $^{15}NH_2OH$.HCl. Yield 93%, m.p. 265–267°C, ^{1}H NMR (DMSO-d_6): δ 6.07 (s, 4H), 7.31 (s, 2H), 9.92 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 153.6, 148.7, 147.9, 127.4, 111.7. MS (ESI) *m/e* (rel. int.): 345 ($M^+ + 1$, 100), 330 (15), 240 (55), 176 (50). High resolution mass calculated for $C_{16}H_9D_6N_6O_3$: 345.1582. Observed 345.1575. (**11** hydrochloride salt); m.p. 285–287°C dec. *Analytical* Calculated for $C_{16}H_8D_6N_6O_3$-4.0HCl-0.8C_2H_5OH: C, 40.11; H + D as H, 4.32; N, 15.95. Found. C, 40.41; H + D as H, 4.26; N, 16.20.

*2,5-Bis[5-(methoxyamidino)-2-pyridyl]furan-d_6 (**12**)*

The same procedure described for **5a** was used, starting with diamidoxime-d_6 **11**. Yield 60%, m.p. 228–229°C, ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 6H), 6.29 (s, 4H), 7.33 (s, 2H). ^{13}C NMR (DMSO-d_6); δ 153.6, 148.9, 148.3, 126.7, 112.0, 60.8. MS (ESI) *m/e* (rel. int.): 373 ($M^+ + 1$, 100), 357 (10), 319 (10), 284 (20). High resolution mass calculated for $C_{18}H_{13}D_6N_6O_3$: 373.1895. Observed 373.1897. (**12** hydrochloride salt); m.p. 230–231.5°C dec. *Analytical* Calculated for $C_{18}H_{12}D_6N_6O_3$-4.0HCl-1.25H_2O-1.0EtOH: C, 40.93; H + D as H, 5.19; N, 14.30. Found. C, 40.97; H + D as H, 4.87; N, 14.00.

*2,5-Bis[5-(methoxyamidino)-2-pyridyl]furan-d_6 (**14**)*

The same procedure described for **5a** was used, starting with diamidoxime **13**,[13] and employing dimethylsulfate-d_6 instead of dimethylsulfate-d_0. Yield 65%, m.p. 236–237°C, ^{1}H NMR (DMSO-d_6): δ 6.29 (s, 4H), 7.32 (s, 2H), 7.96 (d, J = 8.4 Hz, 2H), 8.11 (dd, J = 8.4, 1.8 Hz, 2H), 8.87 (d, J = 1.8 Hz, 2H).

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^{13}C NMR (DMSO-d_6); δ 153.6, 148.9, 148.3, 147.0, 134.2, 126.9, 118.1, 112.0. MS (ESI) *m*/*e* (rel. int.): 373 ($M^+ + 1$, 100), 225 (25). High resolution mass calculated for $C_{18}H_{13}D_6N_6O_3$: 373.1895. Observed 373.1892. (**14 hydrochloride salt**); m.p. 205–206°C dec. *Analytical* Calculated for $C_{18}H_{12}D_6N_6O_3$-4.0HCl-2.0H_2O: C, 39.00; H + D as H, 4.69; N, 15.15. Found. C, 38.98; H + D as H, 4.65; N, 14.99.

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