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# SYNTHESIS OF DEUTERATED-BCX-34 (PELDESINE)

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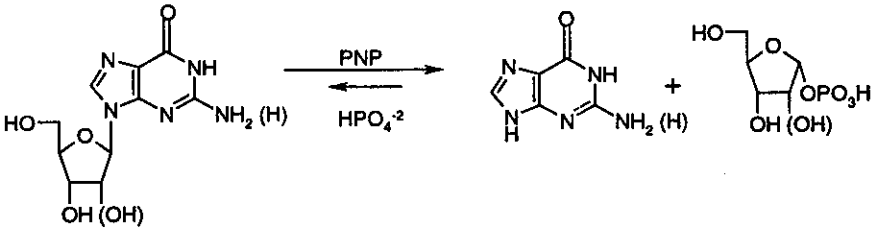
## SUMMARY

BCX-34 (peldesine) is a novel purine nucleoside phosphorylase inhibitor that is in human clinical trials for the treatment of T-cell cancers and for HIV infected patients. In support of our BCX-34 clinical program, a mass spectrometric assay has been developed utilizing a deuterated-BCX-34 analog as an internal standard. The synthesis of tetra-deuterated-BCX-34 (peldesine) from ethyl nicotinate (2,4,5,6-$[^2H]_4$) is described in this report.

**KEY WORDS:** BCX-34, Peldesine, Purine Nucleoside Phosphorylase.

## INTRODUCTION

The enzyme purine nucleoside phosphorylase (PNP, EC 2.4.2.1) catalyzes the reversible cleavage of purine nucleosides to the corresponding purine base and sugar phosphate in the purine salvage pathway as shown below.[1]

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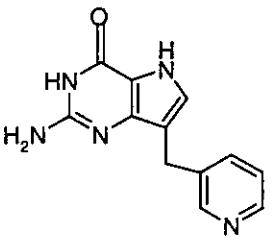
In the absence of the PNP enzyme, the PNP nucleoside substrates inosine, 2'-deoxyinosine, guanosine and 2'-deoxyguanosine (dGuo) accumulate since they are not catabolized. This accumulation of nucleosides has been observed in children with inherited PNP deficiency.[2] These children exhibit severe T-cell immunodeficiency but retain normal or exaggerated B-cell function. From this elevated nucleoside pool, only 2'-deoxyguanosine has an effect on T-cells.[3] The elevated levels of dGuo become rapidly phosphorylated to the monophosphate (dGMP) by 2'-deoxycytidine kinase (EC 2.7.1.74). dGMP is further phosphorylated to the corresponding triphosphate (dGTP) which accumulates in the T-cell since in its highly charged state it cannot cross the cell membrane. dGTP allosterically inhibits the enzyme ribonucleotide diphosphate reductase (EC 1.17.4.1) which shuts down DNA synthesis and hence T-cell proliferation.[4] This observation has led to the development of PNP inhibitors for the treatment of T-cell proliferative diseases such as cutaneous T-cell lymphoma (CTCL) and acute lymphoblastic leukemia (ALL). Autoimmune diseases such as psoriasis, rheumatoid arthritis and Crohn's disease are also believed to be T-cell mediated and should also be amenable to treatment with PNP inhibitors. The biochemical basis for the use of PNP inhibitors as well as the various classes of inhibitors developed has been recently reviewed.[5]

Using structure-based drug design, we have developed several classes of novel PNP inhibitors.[6-8] One of these inhibitors, BCX-34 (peldesine, **1**) is currently in human clinical trials for the treatment of CTCL, ALL and HIV infection. In support of our clinical program, we needed to develop a rapid and sensitive method of determining drug levels in biological matrices such as plasma and urine. One method evaluated for this purpose was isotope dilution selected ion monitoring mass spectrometry (ID-SIM-MS). In this technique an isotopically - labeled analog may be used as an internal standard. In this paper, we present the synthesis of a tetra-deuterated analog of BCX-34 (**2**), which we have used in these assays.



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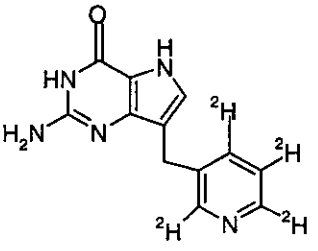

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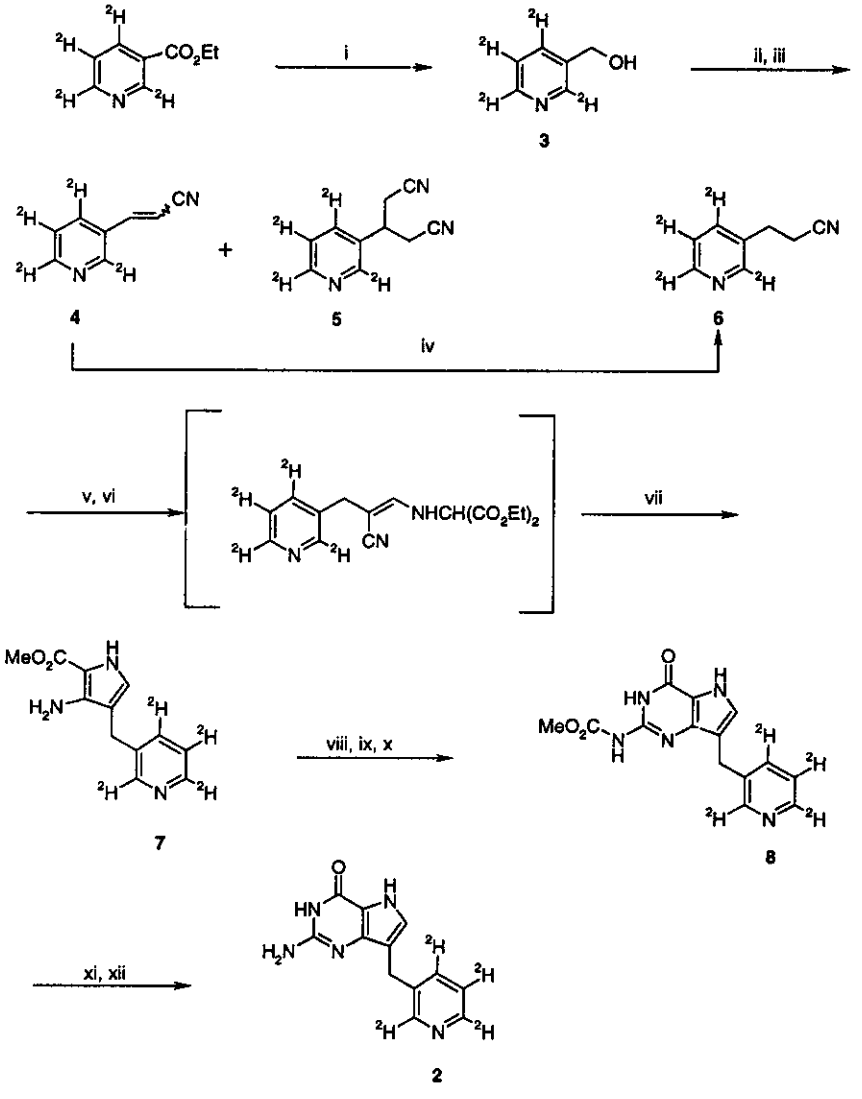
## RESULTS AND DISCUSSION

The synthesis of title compound **2** was based on procedures described earlier by our group for similar 7-substituted pyrrolo[3,2-*d*]pyrimidines.[9] As shown in Scheme 1, commercially available ethyl nicotinate (2,4,5,6-[$^2$H]$_4$) was reduced with $LiAlH_4$ to yield alcohol **3** in 94% yield. Swern oxidation of **3** afforded the corresponding aldehyde in high yield. Attempts were made to synthesize the aldehyde directly from ethyl nicotinate (2,4,5,6-[$^2$H]$_4$) with sodium bis(2-methoxyethoxy)aluminum hydride according to a published procedure.[10] However, in our hands this reduction consistently produced the alcohol as the major product. Knoevenagel condensation of the aldehyde with cyanoacetic acid in a mixture of toluene/pyridine gave a mixture of **4** (19%) and the bis-nitrile **5** (60%) which was purified by flash chromatography. Presumably, the undesired bis-nitrile **5** was formed via a Michael addition of a second cyanoacetic acid moiety followed by decarboxylation. We were unable to prevent the formation of **5** even by limiting the amount of cyanoacetic acid used. This contrasts sharply with the reaction of the unlabled aldehyde, which under identical conditions gives only trace amounts of the bis-nitrile. [11] The difference in the ratios of **4**:**5** with unlabeled versus deuterium labeled substrate is presumably due to a secondary isotope effect, the origin of which is not immediately apparent. Further examination of this phenomenon was outside the scope of the study.

**SCHEME 1**[a]




[a]Reagents: (i) $LiAlH_4$; (ii) oxalyl chloride, TEA, DMSO; (iii) cyanoacetic acid; (iv) $H_2$, Pd/C; (v) ethyl formate, NaH; (vi) diethylaminomalonate hydrochloride; (vii) NaOMe, MeOH; (viii) 1,3-dimethoxycarbonyl-*O*-methylisourea; (ix) NaOMe; (x) HOAc; (xi)NaOH (xii)HOAc

Catalytic hydrogenation of **4** in MeOH gave the propionitrile **6**. The propionitrile **6** was formylated with NaH – ethyl formate and the desired sodium enolate was isolated quantitatively by precipitation with hexane. Condensation of the enolate with diethyl aminomalonate hydrochloride gave the corresponding

enamine which was conveniently converted *in situ* to the pyrrole **7** with NaOMe. Condensation of **7** with 1,3-dimethoxycarbonyl-*O*-methylisourea[12] under mild acidic conditions produced the corresponding protected guanidine adduct which was immediately cyclized *in situ* with NaOMe to the protected pyrrolo[3,2-*d*]pyrimidine derivative **8** in 86% overall yield from pyrrole **7**. Removal of the final carbamate group with aqueous NaOH at 65 °C provided compound **2** in 80% yield. Isotopic purity of **2** as determined by MS analysis was 99.7 %. The overall yield for **2** from ethyl nicotinate (2,4,5,6-[$^2$H]$_4$) was 6.7 % with an isotopic purity of 99.7 %.

## EXPERIMENTAL

**General.** Melting points were determined on a Meltemp II melting point apparatus and are uncorrected. The $^1$H NMR spectra were recorded on a Bruker AMX-360 at 360 MHz or a Bruker AMX-500 at 500 MHz spectrometer. The $^2$H NMR spectra were recorded on a Bruker AMX-500 spectrometer at 76 MHz. The $^{13}$C NMR spectra were recorded on a Bruker AMX-360 at 90 MHz or a Bruker AMX-500 at 125 MHz spectrometer. Chemical shifts (ppm) are referenced to internal tetramethylsilane for $^1$H and $^{13}$C spectra and either $CDCl_3$ ($\delta$ = 7.27) or DMSO ($\delta$ = 2.52) for $^2$H spectra. IR spectra were obtained on a Bio-Rad FTS-7 FT-IR. Mass spectra were recorded on a Fisons VG Trio 2000 in the positive electrospray mode with a scan range of 110-650 amu and cone voltage setting of 30 V. A solution of the sample ($\cong$ 100 μg/ mL) in acetonitrile:$H_2O$:formic acid (50:49:1) was introduced into the source via a syringe pump (10 μL/ min). Flash chromatographic separations were performed on Whatman silica gel, 60 Å. Thin layer chromatography (TLC) was performed using aluminum backed silica gel 60 plates from E. Merck. Ethyl nicotinate (2,4,5,6-[$^2$H]$_4$) was obtained from Cambridge Isotope Laboratories, Inc.

**3-Hydroxymethylpyridine (2,4,5,6-[$^2$H]$_4$) (3):** $LiAlH_4$ (1.0 M in THF, 50 mL, 50 mmol) was added dropwise to a solution of ethyl nicotinate (2, 4, 5, 6-[$^2$H]$_4$) (15.0 g, 96.7 mmol) in dry THF (120 mL) under a $N_2$ atmosphere. The reaction was stirred

at room temperature for 1.5 hours. The reaction was quenched with $H_2O$ (≅ 10 mL), NaOH (2 N, ≅10 mL) followed by $H_2O$ (≅ 5 mL). The precipitate was filtered and the filtrate concentrated *in vacuo* to give **3** as a yellow oil: 10.2 g (94%). IR (neat) 3500-3100 (OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 4.10 (br s, 1 H, O*H*, $D_2O$ exchangeable), 4.63 (s, 2 H, C$H_2$); $^2H$ NMR ($CDCl_3$) δ 7.25 (d, 1 $^2H$), 7.74 (s, 1 $^2H$), 8.45 (d, 2 $^2H$); $^{13}C$ NMR ($CDCl_3$) δ 123.0 (t, $J$ = 24.9 Hz), 134.7 (t, $J$ = 24.3 Hz), 136.8, 147.6 (t, $J$ = 27.0 Hz), 147.8 (t, 27.5 Hz); MS (ES) $m/z$ 114.16 $(M + H)^+$ (100 %).

**3-(3-Pyridinyl)-2-propenenitrile (2,4,5,6-[$^2H$]$_4$) (4)**: A solution of $CH_2Cl_2$ (17 mL) and oxalyl chloride (2 *N* in $CH_2Cl_2$ , 35 mL, 70 mmol) was placed in a three neck round bottom flask equipped with a mechanical stirrer and dropping funnel under a $N_2$ atmosphere. This mixture was cooled to -78 °C (dry ice - acetone) and a solution of DMSO (9.8 mL, 138 mmol) in $CH_2Cl_2$ (32 mL) was added dropwise within 5 minutes. The reaction was stirred for 2 minutes and a solution of **3** (7.1 g, 62.8 mmol) in $CH_2Cl_2$ (43 mL) was added within 5 minutes. The reaction was stirred for an additional 15 minutes and triethylamine (44.5 mL, 320 mmol) was added. After 5 minutes, the reaction was allowed to warm to room temperature and quenched with $H_2O$ (10 mL). The layers were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2 x 100 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and concentrated *in vacuo* to give 3-pyridinecarboxaldehyde as an orange oil (7.4 g) which was used with no additional purification.

A mixture of the above product (7.4 g), cyanoacetic acid (4.5g, 52.9 mmol), ammonium acetate (192 mg, 2.49 mmol), toluene (46 mL) and pyridine (25 mL) was refluxed for 16 h in a flask fitted with a Dean-Stark trap and condenser. The solvents were removed *in vacuo* to yield a dark oil (7.1 g) which was separated by chromatography (450 g $SiO_2$, 50% EtOAc - hexane) to give **4** (1.6 g, 19%) mp 94 - 95 °C; IR (KBr) 2243 $cm^{-1}$ (-CN stretch); $^1H$ NMR ($CDCl_3$) δ 5.97 (d, 1 H, C=C, $J$ = 16.7), 7.41 (d, 1 H, C=C, $J$ = 16.7 Hz); $^2H$ NMR ($CDCl_3$) δ 7.41 (s, 1 $^2H$), 7.83 (s, 1 $^2H$), 8.72

(s, 2 $^2$H); $^{13}$C NMR ($CDCl_3$) δ 98.9, 117.3, 123.3, 129.2, 133.1, 146.9, 148.6, 151.5; MS (ES) *m/z* 135.31 $(M + H)^+$ (100 %).

Further elution gave **5** (4.2 g, 59 %) mp 67-68 °C; IR (KBr) 2252.13 $cm^{-1}$ (-CN stretch); $^1$H NMR ($CDCl_3$) δ 2.84 (d, 4 H, *J* = 13.5 Hz), 3.43 (m, 1 H); $^2$H NMR ($CDCl_3$) δ 7.45 (s, 1 $^2$H), 7.76 (s, 1 $^2$H), 8.65 (s, 2 $^2$H); $^{13}$C NMR ($CDCl_3$) δ 22.68, 36.05, 116.40, 123.45 (t, 1 C, *J* = 25.1 Hz), 133.32, 133.65 (t, 1 C, *J* = 24.5 Hz), 148.17 (t, 1 C, *J* = 27.0 Hz), 149.75 (t, 1 C, *J* = 27.4 Hz); MS (ES) *m/z* 175.96 $(M + H)^+$ (100 %).

**3-(3-Pyridinyl)propanenitrile (2,4,5,6-[$^2$H]$_4$) (6)**: Pd/C (10%, 600 mg) was added to a solution of **4** (1.5 g, 11.2 mmol) in methanol (50 mL). This mixture was hydrogenated at 40 psi $H_2$ for 16 h. The catalyst was filtered through celite and the filtrate was concentrated *in vacuo* to yield **6** as an orange oil: (1.36 g, 89.5 %). IR (neat) 2228.43 $cm^{-1}$ (-CN stretch); $^1$H NMR ($CDCl_3$) δ 2.60 (t, 2 H, *J*= 7.3 Hz), 2.98 (t, 2 H, *J* = 7.3 Hz); $^2$H NMR ($CDCl_3$) δ 7.65 (s, 1 $^2$H), 8.07 (s, 1 $^2$H), 8.80 (s, 2 $^2$H); $^{13}$C NMR ($CDCl_3$) δ 24.73, 101.02, 113.02, 115.73, 145.26, 166.50, 167.51; MS (ES) *m/z* 137.45 $(M + H)^+$ (100 %).

**3-Amino-2-methoxycarbonyl-4-{2,4,5,6-[$^2$H]$_4$-(3-pyridinylmethyl)}-1*H*-pyrrole (7)**: NaH (360 mg, 15 mmol) was slowly added in portions to a solution of **6** (1.36 g, 10 mmol) in THF (20 mL). Ethyl formate (0.1 mL, 1.2 mmole) was added to initiate the reaction. After 15 min, the remaining ethyl formate (2.4 mL, 29.7 mmol) was added dropwise and the mixture stirred at room temperature for 16 h.

During the second day, the remaining NaH (360 mg, 15 mmol) was added in three equal portions while ethyl formate (2.5 mL, 30.9 mmol) was added dropwise. This mixture was again stirred overnight. The reaction mixture was diluted with hexane (30 mL) and the solid product collected by filtration, washed with hexane (10 mL), ether (10 mL) and then partially dried to give the sodium enolate (2.0 g, assumed quantitative yield).

Diethyl aminomalonate hydrochloride (2.5 g, 11.8 mmol) was added to a solution of the sodium enolate from above in methanol (15 mL) and $H_2O$ (5.5 mL). This mixture was stirred at room temperature for 2 days. The methanol was removed *in vacuo* and the residue diluted with water (25 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$) and concentrated *in vacuo* to yield the enamine (2.86 g, 89.1 %) which was used with no further purification.

NaOMe (25%, 2.0 mL, 8.75 mmol) was added slowly to a mixture of the above product (2.43 g, 7.56 mmol) in MeOH (20 mL) and stirred at room temperature for 45 min. The MeOH was removed *in vacuo* and the residue treated with water (20 mL) to give **7** (1.08 g, 61 %) mp 121-122 °C; IR (KBr) 3462.38, 3368.05, 1665.68, 1609.51 $cm^{-1}$; $^1H$ NMR (DMSO) δ 3.32 (s, 2 H), 3.68 (s, 3 H), 4.91 (br s, 2 H, $D_2O$ exchangeable), 6.53 (s, 1 H), 10.51 (br s, 1 H, $D_2O$ exchangeable); $^2H$ NMR (DMSO) δ 7.30 (s, 1 $^2H$), 7.62 (s, 1 $^2H$), 8.39 (s, 2 $^2H$); $^{13}C$ NMR ($CDCl_3$) δ 26.34, 49.99, 104.63, 109.61, 123.03, 135.33 (t, 1 C, $J$ = 24.7 Hz), 136.68, 140.73, 146.47 (t, 1 C, $J$ = 26.3 Hz), 149.21 (t, 1 C, $J$ = 26.4 Hz), 161.06, 161.39; MS (ES) $m/z$ 236.48 $(M + H)^+$ (100 %).

**1,5-Dihydro-2-(methoxycarbonylamino)-7-{(2,4,5,6-[$^2H$]$_4$-(3-pyridinylmethyl)} -4*H*-pyrrolo[3,2-*d*]pyrimidin-4-one (8):** 1,3-Dimethoxycarbonyl-*O*-methylisourea (1.6g, 8.4 mmol) was added to a solution of **7** (1.6 g, 6.8 mmol) in MeOH/HOAc (10 mL/ 1.95 mL) and stirred at room temperature for 16 h. TLC analysis with 10% MeOH-$CHCl_3$ showed the presence of some starting material. Additional 1,3-dimethoxycarbonyl-*O*-methylisourea (904 mg, 4.8 mmol) and HOAc (0.8 mL) was added and the reaction mixture warmed to 40 °C for 24 h during which time the reaction went to completion. NaOMe (25%, 12.6 mL, 55 mmol) was added to the reaction mixture and stirred at room temperature for 6 h. HOAc (0.4 mL) was added to neutralize the base. The solid was collected by filtration, washed with $H_2O$ (30 mL) and dried overnight to yield **8** (1.77 g, 86%) as an off-white solid. mp >226 °C

(dec); IR (KBr) 3219, 1714, 1677, 1622; $^1$H NMR (DMSO-d$_6$) δ 3.70 (s, 3 H), 3.89 (s, 2 H), 7.15 (s, 1 H), 11.42 (br s, 3 H, $D_2O$ exchangeable); $^2$H NMR (DMSO-d$_6$) δ 7.29 (s, 1 $^2$H), 7.67 (s, 1 $^2$H), 8.42 (s, 1 $^2$H), 8.53 (s, 1 $^2$H); $^{13}$C NMR (DMSO-d$_6$) δ 26.47, 52.52, 113.86, 114.41, 122.73 (t, 1 C, $J$ = 23.9 Hz), 126.37, 135.31 (t, 1 C, $J$ = 23.9 Hz), 136.71, 142.71, 144.02, 146.51 (t, 1 C, $J$ = 25.7 Hz), 149.07 (t, 1 C, $J$ = 26.7 Hz), 152.63, 155.65; MS (ES) $m/z$ 304.50 $(M + H)^+$ (100 %).

**2-Amino-1,5-dihydro-7-{(2,4,5,6-[$^2$H]$_4$-(3-pyridinylmethyl)}-4*H*-pyrrolo[3,2-*d*]-pyrimidin-4-one (2):** Compound **8** (600 mg, 1.98 mmol) was added to a solution of NaOH (1.07 g) in $H_2O$ (15 mL) and warmed at 65 °C for 12 hours. TLC ($CHCl_3$–MeOH-$NH_4OH$ 80:18:2) analysis showed complete conversion of **8** to **2**. The solution was filtered and the filtrate was neutralized with HOAc. The solid was collected by vacuum filtration, washed with $H_2O$ (30 mL) and dried under vacuum to afford **2**: (389 mg, 80.2%) as an off-white powder, mp >281 °C (dec); $^1$H NMR (DMSO-d$_6$) δ 3.81 (s, 2 H), 5.81 (s, 2 H, $D_2O$ exchangeable), 6.96 (s, 1 H), 10.35 (s, 1 H, $D_2O$ exchangeable), 11.24 (s, 1 H, $D_2O$ exchangeable); $^2$H NMR (DMSO-d$_6$) δ 7.29 (s, 1 $^2$H), 7.84 (s, 1 $^2$H), 8.38 (s, 1 $^2$H), 8.51 (s, 1 $^2$H); $^{13}$C NMR (DMSO-d$_6$) δ 26.55, 112.49, 112.54, 122.66 (t, 1 C, $J$ = 24.5 Hz), 125.44, 135.22 (t, 1 C, $J$ = 25,1 Hz), 137.14, 145.09, 146.39 (t, 1 C, $J$ = 26.7 Hz), 149.08 (t, 1 C, $J$ = 26.7 Hz), 150.42, 154.06; MS (ES) $m/z$ 246.02 $(M + H)^+$ (100 %).

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11. Parallel reaction of unlabeled pyridine-3-carboxaldehyde gave > 80% of unlabeled **4**. None of the bis-nitrile **5** was observed. We have repeated this reaction many times up to the multi-kilo scale with similar results.

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