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Novel M_4 positive allosteric modulators derived from questioning the role and impact of a presumed intramolecular hydrogen-bonding motif in β-amino carboxamide-harboring ligands

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ABSTRACT

This letter describes a focused exercise to explore the role of the β-amino carboxamide moiety found in all of the first generation M_4 PAMs and question if the NH_2 group served solely to stabilize an intramolecular hydrogen bond (IMHB) and enforce planarity. To address this issue (and to potentially find a substitute for the β-amino carboxamide that engendered P-gp and contributed to solubility liabilities), we removed the NH_2, generating *des*-amino congeners and surveyed other functional groups in the β-position. These modifications led to weak M_4 PAMs with poor DMPK properties. Cyclization of the β-amino carboxamide moiety by virtue of a pyrazole ring re-enforced the IMHB, led to potent (and patented) M_4 PAMs, many as potent as the classical bicyclic β-amino carboxamide analogs, but with significant CYP1A2 inhibition. Overall, this exercise indicated that the β-amino carboxamide moiety most likely facilitates an IMHB, and is essential for M_4 PAM activity within classical bicyclic M_4 PAM scaffolds.

Positive allosteric modulators (PAMs) of the muscarinic acetylcholine receptor subtype 4 (M_4) have garnered a great deal of attention and interest as next generation antipsychotics via a novel mechanism of action.[1–18] From the first account of an M_4 PAM ligand, **1**, the β-amino carboxamide moiety (circled in red) was present, and this functionality was maintained through multiple iterations of advanced ligands **2–5** (either thieno[2,3-*b*]pyridine 2-carboxamides, e.g., **1** and **2**, or thieno [2,3-*c*]pyridazines 6-carboxamides, e.g., **3–5**) enroute to preclinical development candidates (Fig. 1).[1–18] While potent and highly selective M_4 PAMs resulted that provided critical *in vivo* target validation and enabled deep mechanistic studies, this chemotype engendered poor physiochemical properties and variable species-specific P-gp efflux liabilities. Only recently have alternative M_4 PAM chemotypes been reported.[19,20] We sought to better understand the role of the β-amino carboxamide moiety, and question if this non-basic 'spectator' functionality served to enforce an intramolecular hydrogen bond (IMHB) and engender planarity.[21] In this Letter, we describe a series of studies and novel ligands that support the IMHB as a crucial pharmacophore for M_4 PAM activity.

To address this IMHB issue (and to potentially find a substitute for the β-amino carboxamide that engendered P-gp and solubility liabilities), we first removed the NH_2, generating *des*-amino congeners as well as ligands with other groups replacing the NH_2 moiety. Second, we re-enforced the planarity of the presumed IMHB with a novel tricycle core, and assessed M_4 PAM activity (Fig. 2). Combined, the necessity of the IMHB and an extended planar disposition was clear.

The synthesis of *des*-amino and alternatively functionalized congeners **6** is shown in Scheme 1 for the thieno[2,3-*b*]pyridine scaffold (similar chemistry was used for thieno[2,3-*c*]pyridazine analogs).[1–18] Condensation of α-bromoacetate with commercial thiopyridone **8** affords **9** in 66% yield. A subsequent Sandmeyer reaction converts the NH_2 to the corresponding bromide **10** in 81% yield. The bromide could then be reduced to afford the *des*-amino derivative **14**, which could then be saponified and coupled with preferred amines to provide analog

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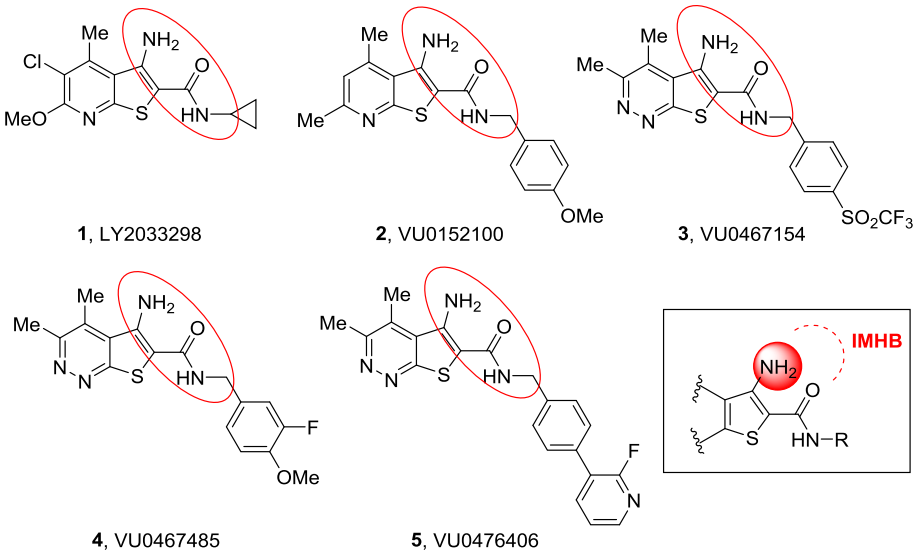


Fig. 1. Prototypical M_4 PAM chemotypes featuring a conserved β-amino carboxamide moiety, presumed to participate in an intramolecular hydrogen bond (IMHB), resulting in an extended planar conformation. However, this presumed essential pharmacophore also engendered poor physiochemical properties and unpredictable P-gp liabilities.

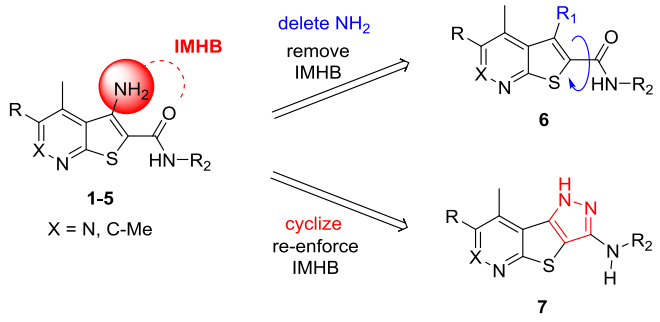


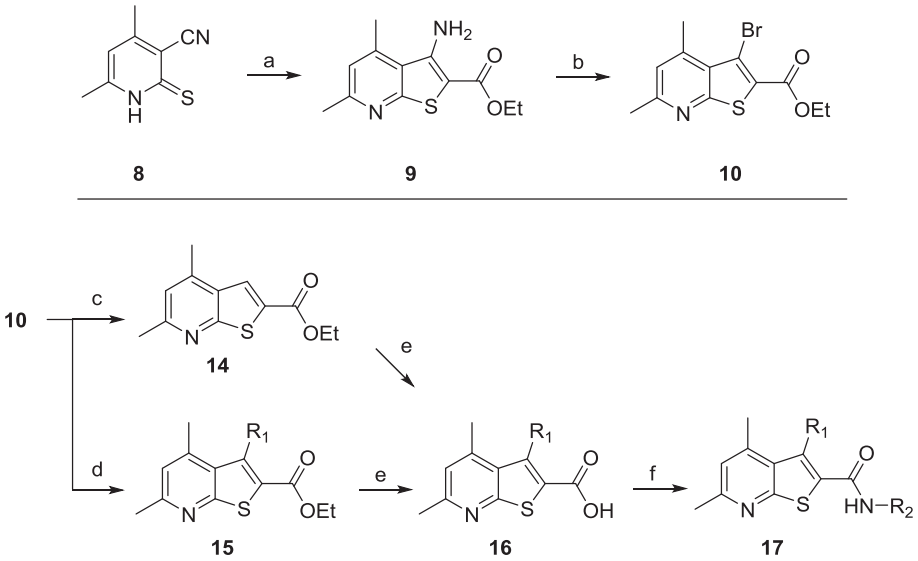
Fig. 2. The prototypical M_4 PAM scaffolds **1–5**, and plans to assess the role of the IMHB, by either removing the β-NH_2 and surveying alternative moieties that remove the IMHB capability as in **6**, or by cyclization through a pyrazole ring, affording tricycles **7**, that reinforce the IMHB.

17a (wherein R_1 = H). Alternatively, **10** could undergo Suzuki coupling reactions to install small alkyl moieties to replace the NH_2 group, followed by ester hydrolysis and amide couplings to deliver analogs **17**.

As shown in Table 1, the classical β-amino carboxamide M_4 PAM **2** is a moderately potent PAM (EC_{50} = 680 nM),[1–18] but removal of the NH_2 to provide **17a** (R_1 = H) leads to a significant diminution in potency (EC_{50} = 5.1 μM). Substitution of the NH_2 for a CH_3 group (**17b**) again loses potency (EC_{50} = 2.7 μM), but improved activity relative to the *des*-amino congener **17a**, suggesting free rotation about the amide bond is detrimental to M_4 PAM activity. Neither alternative, preferred amides nor larger alkyl groups (e.g., ethyl **17h**) could improve M_4 PAM potency to that of the β-amino carboxamide-based ligands.[1–18]

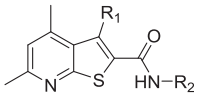
As the thieno[2,3-*c*]pyridazine core was more potent than the thieno[2,3-*b*]pyridine scaffold amongst the classical β-amino carboxamide M_4 PAMs,[1–18] we also evaluated derivatives **18** (Table 2). Here, parent NH_2-baring **4** was a potent M_4 PAM (EC_{50} = 84 nM), but the *des*-amino analog **18a** lost ~18-fold potency (EC_{50} = 1.5 μM). Methyl (**18b**), ethyl (**18c**) and cyclopropyl (**18d**) congeners were all of similar, weak M_4 PAM potency, indicating the necessity of the IMHB and an extended planar disposition through the β-amino carboxamide. Moreover, all congeners **17** and **18** displayed inferior DMPK properties (e.g., high predicted hepatic clearance (rat CL_{hep} > 68 mL/min/kg), high protein binding (rat $f_{u,plasma}$ < 0.005) and mixed CYP_{450} inhibition profiles) as compared to **2–5**. Thus, efforts shifted to focus on the generation of three distinct tricyclic cores, each incorporating a pyrazole to mimic the β-amino carboxamide, to re-enforce the IMHB.

Synthesis of the first desired tricyclic core, a 1*H*-pyrazolo[3′,4′:4,5]thieno[2,3-*b*]pyridine, was accomplished in four linear steps (Scheme

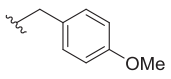
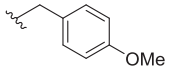
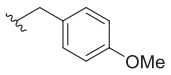
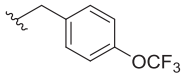
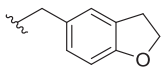
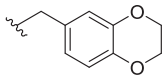
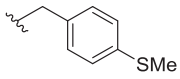
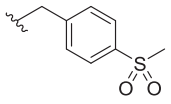
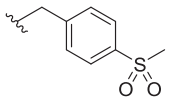


Scheme 1. Synthesis of *des*-methyl analogs **17** of **6**. Reagents and conditions: (a) α-bromoethyl acetate, aq. KOH, DMF, rt, 66%; (b) $CuBr_2$, *t*-BuONO, CH_3CN, 65 °C, 81%; (c) Bu_3P, $Pd(OAc)_2$, Et_3N, HCOOH, DMF, 100 °C, 45%; (KOH, EtOH/H_2O, 65 °C, 85–90%; (d) KF_3B-R_1, $PdCl_2(dppf)CH_2Cl_2$, Cs_2CO_3, THF/H_2O, 140 °C mw, 60–67%; (e) KOH, EtOH/H_2O, rt, 85–90%; (f) NH_2R_2, HATU, DIEA, DMF, rt, 15–25%.

Table 1
Structure and hM_4 PAM Activities of analogs **17**.

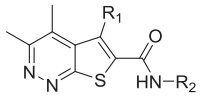


17

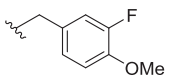
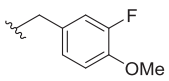
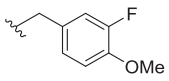
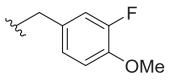
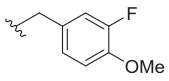
Cmpd	R_1	R_2	hM_4 EC_{50} (μM)[a] [% ACh Max]
2	NH_2		0.68 ± 0.01 [89 ± 5]
17a	H		5.1 ± 1 [53 ± 0.3]
17b	CH_3		2.7 ± 0.3 [49 ± 5]
17c	CH_3		> 10 [29 ± 1]
17d	CH_3		5.9 ± 2 [50 ± 2]
17e	CH_3		2.1 ± 0.3 [56 ± 4]
17f	CH_3		1.5 ± 0.3 [45 ± 3]
17 g	CH_3		6.4 ± 1 [58 ± 2]
17 h	CH_2CH_3		2.7 ± 0.3 [57 ± 2]

[a] For SAR determination, calcium mobilization human M_4/G_{qi5} assays were performed n = 1 independent times in triplicate with an EC_{20} fixed concentration of acetylcholine.

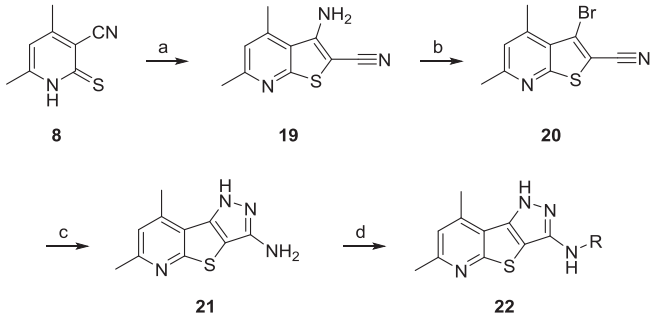
Table 2
Structure and hM_4 PAM activities of analogs **18**.



18

Cmpd	R_1	R_2	hM_4 EC_{50} (μM)[a] [% ACh Max]
4	NH_2		0.08 ± 0.01 [75 ± 2]
18a	H		1.5 ± 0.07 [66 ± 0.2]
18b	CH_3		2.1 ± 0.03 [56 ± 2]
18c	CH_2CH_3		2.2 ± 0.5 [73 ± 2]
18d			5.6 ± 1 [35 ± 1]
18e			> 10 [38 ± 3]

[a] For SAR determination, calcium mobilization human M_4/G_{qi5} assays were performed n = 1 independent times in triplicate with an EC_{20} fixed concentration of acetylcholine.



8 **19** **20** **21** **22**

Scheme 2. Synthesis of tricyclic analogs **22** of **7**. Reagents and conditions: (a) 2-chloroacetonitrile, 10% KOH, DMF, 96%; (b) $CuBr_2$, *t*-BuONO, CH_3CN, 60 °C, 65%; (c) hydrazine, DMSO, 150 °C mw, 98%; (d) RCHO, $NaB(OAc)_3H$, AcOH, $ClCH_2CH_2Cl$, 73–90%.

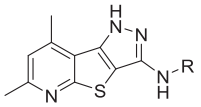
2). Starting once again from thiopyridone **8**, a condensation with 2-chloroacetonitrile provides bicycle **19** in 96% yield. Next, our standard Sandmeyer conditions give bromide **20**, which upon treatment with hydrazine under microwave heating delivers the desired amino tricycle **21** in 98% yield. Finally, a reductive amination protocol affords the first of three sets of tricyclic analogs **22** for evaluation as M_4 PAMs.

Relative once again to M_4 PAM **2**,[1–18] analogs **22** were less potent, but by less than 3-fold – an encouraging find for such a significant change in structure (Table 3). For example, the direct analog of **2**, PAM **22a** was a moderately potent M_4 PAM (EC_{50} = 2.2 μM); however, this modification led to a poor *in vitro* DMPK profile (**22a**: CL_{hep} = 66 mL/min/kg, rat f_u < 0.002, CYP_{450} < 5 μM). These data led us to consider two modifications: 1) a more highly functionalized pyridine core (known historically to improve PAM potency) and 2) cap of the NH of the pyrazole in hopes of engendering CNS penetration.

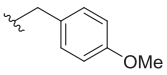
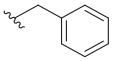
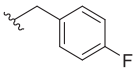
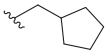
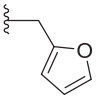
Scheme 3 shows the route to access the more highly substituted and *N*-Me-pyrazole capped tricyclic variants **27**. The chemistry follows that of **22** up to intermediate **25**. Condensation of **25** with methylhydrazine, under microwave irradiation, provides the tricycle in 95% yield. Once again, a final reductive amination protocol affords fully elaborated tricyclic analogs **27** for evaluation as M_4 PAMs. As shown in Table 4, initial SAR was disappointing, as classical, preferred benzyl amide analogs **27a-c** were inactive (EC_{50}s > 10 μM). Interestingly, a cyclopropyl methyl derivative **27d** proved to be a potent M_4 PAM (EC_{50} = 230 nM), as did a furyl methyl congener **27e** (EC_{50} = 120 nM), and equipotent to first generation bicyclic PAMs **1** and **2**. While exciting, **27d** and **27e** suffered poor, non-advanceable DMPK profiles (rat predicted CL_{hep} > 67 mL/min/kg, rat $f_{u,plasma}$s < 0.001) and potent CYP450 inhibition, particularly at 1A2 (IC_{50}s < 100 nM) and 2C9 (IC_{50}s ~ 1.0 μM). Thus, tricycle **27** offered mixed results. On the one hand, an *N*-Me tricycle restored M_4 PAM potency and supported the role of the IMHB in **1**–**5**,[1–18] while, on the other hand, these novel cores suffer from poor disposition, precluding advancement.

Lastly, we wanted to explore a pyridazine-based tricycle, e.g., a 1*H*-pyrazolo[3′,4′:4,5]thieno[2,3-*c*]pyridazine core, as classical M_4 PAMs **3**–**5** possessed this heterocycle and had advanced the farthest in

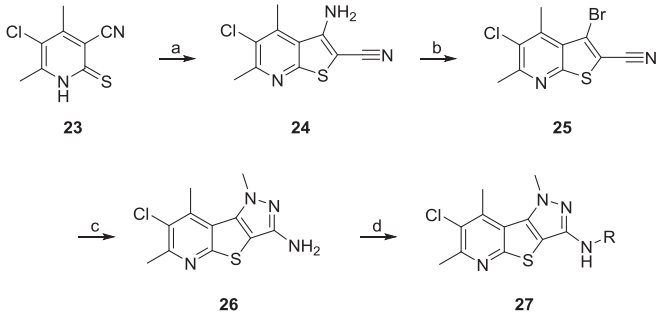
Table 3
Structure and hM_4 PAM activities of analogs **22**.



22

Cmpd	R	hM_4 EC_{50} (μM)[a] [% ACh Max]
22a		2.2 ± 0.4 [67 ± 1]
22b		1.0 ± 0.1 [66 ± 2]
22c		1.1 ± 0.1 [62 ± 2]
22d		2.0 ± 0.5 [67 ± 3]
22e		3.8 ± 0.8 [65 ± 3]
22f		1.2 ± 0.2 [76 ± 5]

[a] For SAR determination, calcium mobilization human M_4/G_{qi5} assays were performed n = 1 independent times in triplicate with an EC_{20} fixed concentration of acetylcholine.



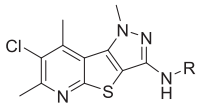
23 **24** **25** **26** **27**

Scheme 3. Synthesis of tricyclic analogs **27** of **7**. Reagents and conditions: (a) 2-chloroacetonitrile, 10% KOH, DMF, 91%; (b) $CuBr_2$, *t*-BuONO, CH_3CN, 60 °C, 75%; (c) methylhydrazine, DMSO, 125 °C mw, 95%; (d) RCHO, $NaB(OAc)_3H$, AcOH, $ClCH_2CH_2Cl$, 73–83%.

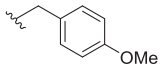
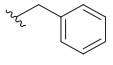
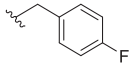
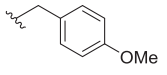
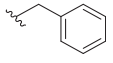
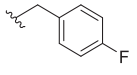
development. To this end, commercial pyridazine **28** was first subjected to an S_N2 reaction with Na_2S, followed by condensation with 2-chloroacetonitrile to deliver **29**. Repetition of the Sandmeyer and methyl hydrazine condensation sequences, followed by reductive amination provides the desired, putative M_4 PAMs **30** (Scheme 4).

As shown in Table 5, tricycle analogs **30** were potent M_4 PAMs, uniformly affording sub-micromolar PAM EC_{50}s. Analog **30a**, harboring the classic PMB amide,[1–18] was a potent M_4 PAM (EC_{50} = 44 nM) with measurable CNS penetration in rat (rat K_p = 0.25), but displayed high predicted hepatic clearance (rat CL_{hep} = 67.8 mL/min/kg) and modest protein binding (rat f_u = 0.02). Another preferred benzyl amide congener, **30b**, was potent (EC_{50} = 160 nM), with improved protein binding (f_u = 0.05), and a clean CYP_{450} profile (IC_{50}s > 30 μM);

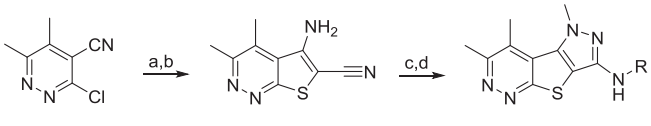
Table 4
Structure and hM_4 PAM activities of analogs **27**.



27

Cmpd	R	hM_4 EC_{50} (μM)[a] [% ACh Max]
27a		> 10 [51 ± 4]
27b		> 10 [39 ± 1]
27c		> 10 [56 ± 3]
27d		0.23 ± 0.02 [67 ± 6]
27e		0.12 ± 0.02 [59 ± 2]
27a		> 10 [51 ± 4]
27b		> 10 [39 ± 1]
27c		> 10 [56 ± 3]
27d		0.23 ± 0.02 [67 ± 6]
27e		0.12 ± 0.02 [59 ± 2]

[a] For SAR determination, calcium mobilization human M_4/G_{qi5} assays were performed n = 1 independent times in triplicate with an EC_{20} fixed concentration of acetylcholine.

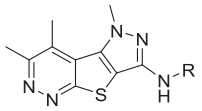


28 **29** **30**

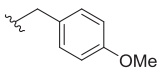
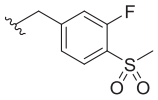
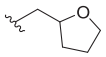
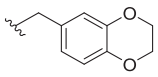
Scheme 4. Synthesis of tricyclic analogs **30** of **7**. Reagents and conditions: (a) i. Na_2S, *t*-BuOH, ii. 2-chloroacetonitrile, NaOAc, EtOH, rt; (b) $CuBr_2$, *t*-BuONO, CH_3CN, 60 °C; (c) methylhydrazine, DMSO, 125 °C mw, 36–58% over 4 steps; (d) RCHO, $NaB(OAc)_3H$, AcOH, $ClCH_2CH_2Cl$, 27–56%.

however, the polarity that improved these DMPK properties led to poor brain penetration in rat (brain concentration BLQ) and rendered **30b** a human P-gp substrate (ER > 10). The remaining analogs **30** also displayed DMPK profiles not suitable for further advancement towards clinical candidates; however, they affirmed the role of the IMHB in **1–5**, and that novel tricycles could be constructed with equipotent M_4 PAM activity. Overall, all three series of tricycles had favorable physiochemical properties (MW < 400, clogPs < 4, CNS MPO scores > 5,[22]

Table 5
Structure and hM_4 PAM activities of analogs **30**.



30

Cmpd	R	hM_4 EC_{50} (μM)[a] [% ACh Max]
30a		0.04 ± 0.003 [79 ± 4]
30b		0.16 ± 0.003 [58 ± 1]
30c		0.62 ± 0.05 [84 ± 3]
30d		0.18 ± 0.02 [102 ± 3]
30e		0.69 ± 0.08 [91 ± 2]
30f		0.06 ± 0.01 [91 ± 3]

[a] For SAR determination, calcium mobilization human M_4/G_{qi5} assays were performed n = 1 independent times in triplicate with an EC_{20} fixed concentration of acetylcholine.

tPSAs 70–90 $Å^2$), suggesting further optimization efforts in this chemical space is warranted.

In summary, we examined the role and impact of a putative intramolecular hydrogen bond amongst the classical series of β-amino carboxamide-based M_4 PAMs. By synthesizing and assessing *des*-amino and alternatively functionalized ligands, the importance of the IMHB was clear. Re-enforcement of the IMHB via novel tricyclic analogs ultimately led to very potent M_4 PAMs and further validation of the IMHB of the β-amino carboxamide moiety as a requisite pharmacophore for M_4 PAM activity. While tricycles allowed entry into novel chemical space[23,24] from which to access M_4 PAMs, disposition was favorable, yet variable. Further efforts based on these findings to develop additional, novel M_4 PAMs are underway, and results will be reported in due course.

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