Supplementary Material (ESI) for Chemical Communications
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The change of corrin-amides to carboxylates leads to altered structures of the B_{12}-responding *btuB* riboswitch†

Electronic Supplementary Information (ESI)

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† Electronic supplementary information (ESI) contains Fig. S1-S4 and Table S1.

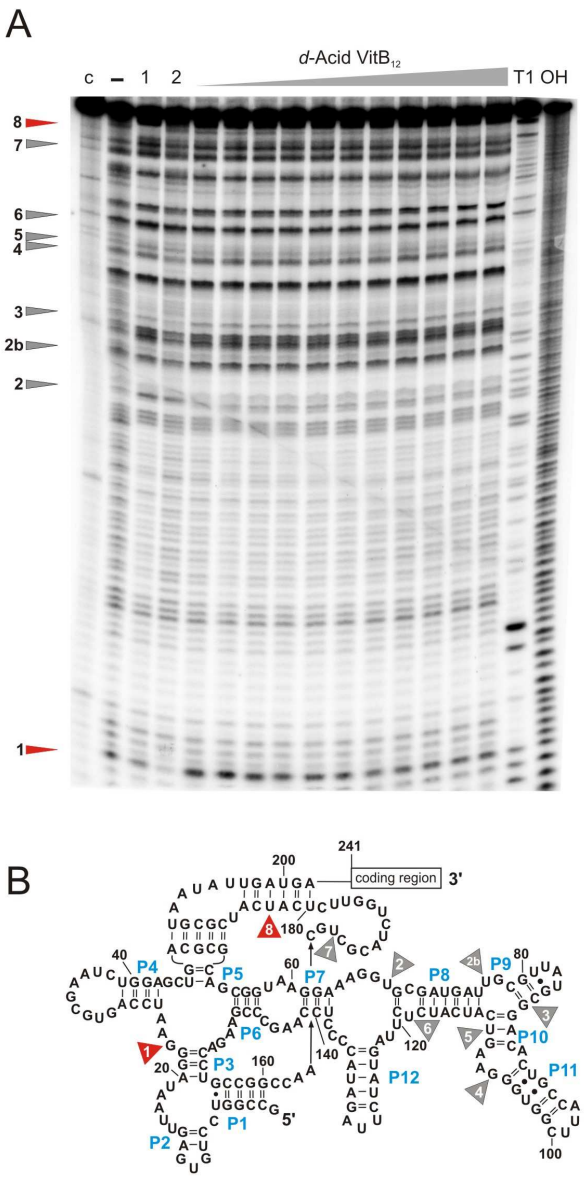

Fig. S1 In-line probing experiments of the *btuB* riboswitch with *d*-acid $VitB_{12}$. (**A**) The structural changes of the RNA induced upon addition of *d*-acid $VitB_{12}$ are similar to the ones induced by *b*-acid $VitB_{12}$ and are limited to sites 1 and 8 (marked in red). The other seven sites (marked in grey) are not affected. (**B**) Cleavage sites mapped onto the proposed secondary structure showing that the full right-hand side of the riboswitch (P8 to P11) remains unaffected by binding to *d*-acid $VitB_{12}$. 7 nM of *btuB* RNA was incubated for 40 hours in 50 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$ and 100 mM KCl in the absence of B_{12} (-) or in the presence of 10 µM, 50 µM, 100 µM, 0.5 mM, 0.75 mM, 1 mM, 2 mM, 4 mM, 5 mM, 7.5 mM and 9 mM of *d*-acid vitamin B_{12}. Lane (c) is the unreacted RNA, T1 the RNAse T1 digestion and OH the alkaline digestion ladder. Lane (1) indicates the *btuB* RNA incubated in the presence of 5 mM $VitB_{12}$ and lane (2) the RNA incubated with 10 µM AdoCbl for comparison.

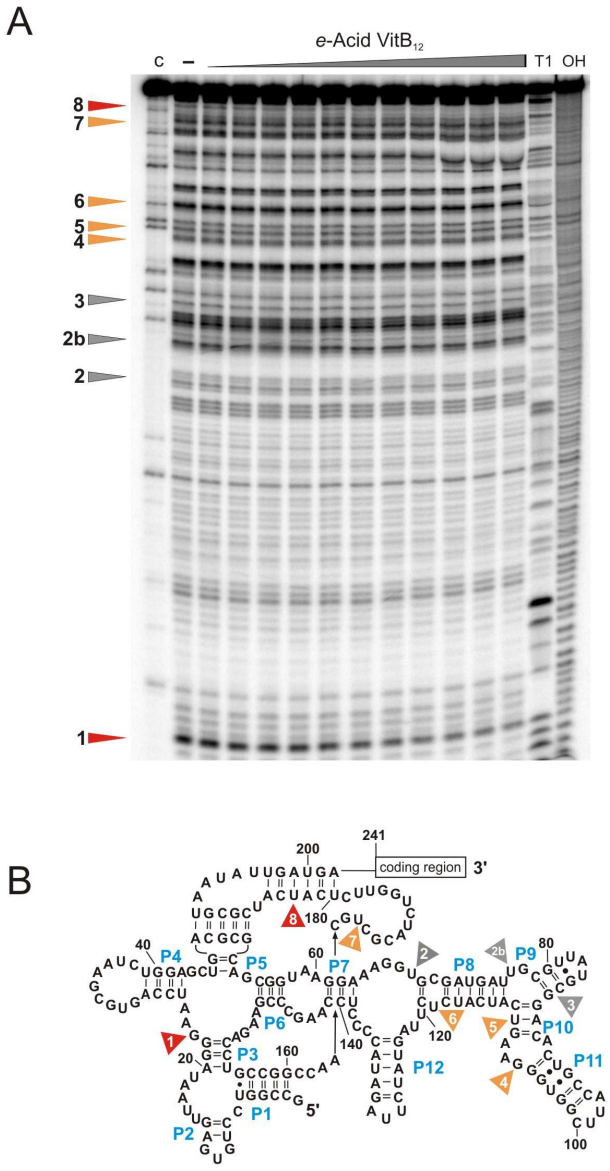

Fig. S2 In-line probing experiments of the *btuB* riboswitch with *e*-acid $VitB_{12}$. (**A**) Addition of *e*-acid $VitB_{12}$ mostly affects sites 1 and 8 (marked in red) although the cleavage intensity is decreased in comparison to incubation with AdoCbl. Intensity changes at sites 4, 5, 6 and 7 (marked in orange) are visible only after background correction but are within the error limits of the experiment. No affinities can be calculated for the latter sites, as the error limits are too large. (**B**) Cleavage sites induced by *e*-acid $VitB_{12}$ mapped onto the proposed secondary structure of the *btuB* RNA. 30 nM of *btuB* RNA was incubated for 41 hours in 50 mM Tris-HCl, pH 8.3, 20 mM $MgCl_2$ and 100 mM KCl in the absence of B_{12} (-) or with 1 µM, 10 µM, 50 µM, 100 µM, 250 µM, 500 µM, 750 µM, 1 mM, 5 mM, 7.5 mM, and 10 mM *e*-acid $VitB_{12}$. Lane (c) is the unreacted RNA, T1 the RNAse T1 digestion and OH the alkaline hydrolysis ladder.

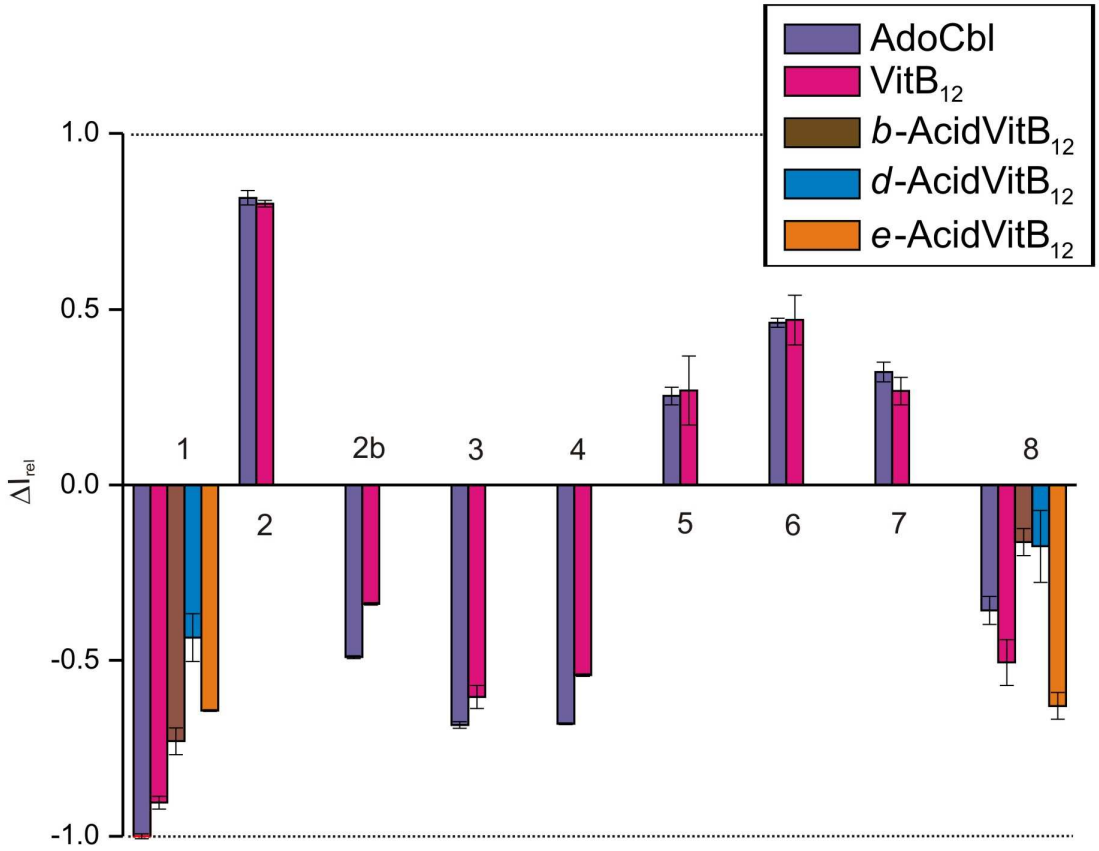

Fig. S3 Relative maximal changes in cleavage intensity at the nine cleavage sites after addition of the three $VitB_{12}$ acids. Comparison with AdoCbl and $VitB_{12}$ shows, that the rearrangement of the *btuB* riboswitch induced by the *b*-, *d*- and *e*- acid derivatives remains limited to the sites 1 and 8. The induced maximal structural changes at site 1 reach 73%, 44 % and 64% compared to the one induced by AdoCbl. The intensity changes induced at site 8 by the three acids diverge more strongly reaching 46%, 49% and 176% from the value induced by incubation with AdoCbl. Changes at the other cleavage sites were either not observed or had too large error limits. All intensity changes shown are given relative to the maximally observed change at site 1 upon addition of AdoCbl. The values depicted correspond to the weighted mean of the results obtained from two or three titration experiments and the error limits shown correspond to one standard deviation.

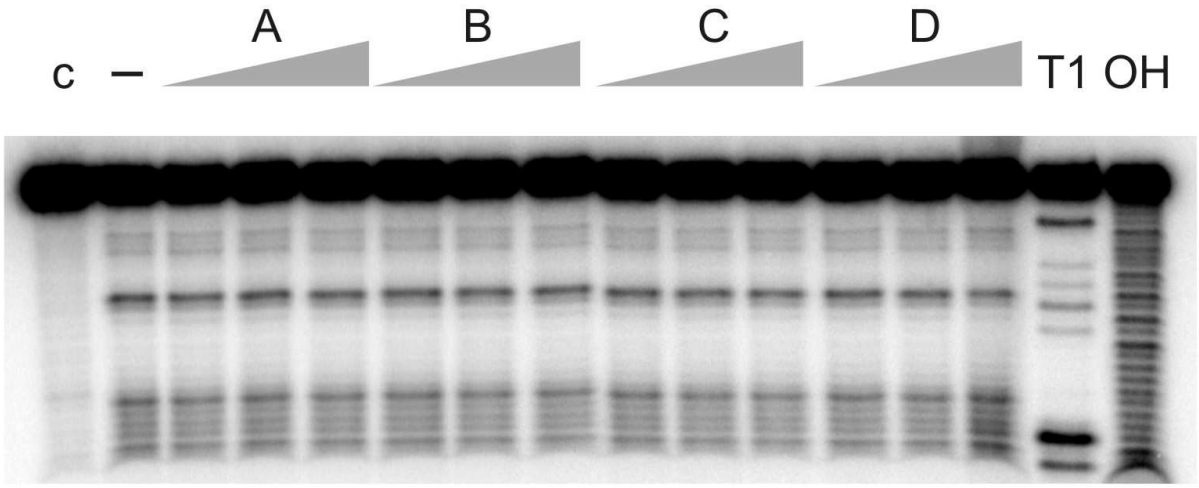

Fig. S4 Titration of κ-ζ RNA with different vitamin B_{12} derivates. The 45 nt long RNA corresponds to the κ-ζ region of the yeast mitochondrial group II intron Sc.ai5γ and has the sequence 5'-GGAGAUAUGCUCAACGAAAGUGAAUCUUCGGAGAGCUAAGUCUCC-3'. The RNA was incubated for 40 h at 25 °C in 50 mM Tris-HCl (pH 8.3 at 25 °C), 20 mM $MgCl_2$ and 100 mM KCl in the absence (-) or in the presence of 10 mM, 100 mM and 1.5 mM of either AdoCbl (A), vitamin B_{12} (B), *b*-acid VitB_{12} (C) or *d*-acid VitB_{12} (D). Lane (c) shows the unreacted RNA, T1 the RNAse T1 digestion and OH the alkaline digestion ladder.

Table S1. The affinities log *K* and the corresponding K_D values for the binding of the four acid derivatives to the *btuB* riboswitch. Given are the values derived from each individual cleavage site as well as the average affinities of each derivative to the riboswitch. For comparison, the left row shows the values obtained for $VitB_{12}$ (*ChemBioChem* **2008**, *9*, 1408-1414). The individual values for each band are the weighted mean of two or three independent experiments and are derived from a 1:1 fit.

site	**log K_{VitB12}**	**log $K_{b\text{-acid VitB12}}$**	**log $K_{d\text{-acid VitB12}}$**	**log $K_{e\text{-acid VitB12}}$**
1	4.09 ± 0.02	2.50 ± 0.04	3.29 ± 0.14	3.56± 0.04
2	3.39 ± 0.22	-	-	-
2b	3.52 ± 0.29	-	-	-
3	3.45 ± 0.08	-	-	-
4	3.47 ± 0.18	-	-	-
5	3.21 ± 0.48	-	-	-
6	3.37 ± 0.31	-	-	-
7	3.40 ± 0.50	-	-	-
8	3.63 ± 0.11	2.71 ± 0.54[b]	3.21 ± 0.37 [a]	3.60 ± 0.04
log K_{av}	**3.50 ± 0.08**	**2.50 ± 0.11** [c]	**3.28 ± 0.04** [c]	**3.58 ± 0.02** [c]
K_D	**316 ± 58 µM**	**3.16 ± 0.80 mM** [c]	**524 ± 48 µM** [c]	**263 ± 12 µM** [c]

[a] Two data sets out of four titrations could be evaluated. [b] Three data sets out of four titrations could be evaluated. [c] Averaged value for sites 1 and 8 only.