Cyclotides are a large family of plant peptides that are characterised by a cystine knot motif together with backbone cyclisation that confers exceptional stability in biological systems. This stability has generated interest in the cyclotide framework as a pharmaceutical scaffold: a potential highlighted by the successful grafting of several bioactive sequences into both Möbius and trypsin inhibitor cyclotides. Backbone cyclisation can also increase the stability and oral availability of bioactive linear peptides suggesting that cyclisation of peptides will find broad application. Elucidating the mechanism of enzymic cyclisation intrinsic to cyclotide biosynthesis is important, not only for the realisation of the pharmaceutical and agricultural potential of cyclotides, but also for increasing the cyclisation efficiency of unrelated, bioactive peptides. Here we report the biochemical features of the cyclotide precursors that are important for cyclotide biosynthesis in *Oldenlandia affinis* and will describe the properties of the asparaginyl endoproteinase (AEP) that produces the prototypic cyclotide, kalata B1. Using this knowledge, we have produced several different gene constructs and have used them to generate transgenic plants that produce cyclotides with up to 100% cyclisation efficiency. We have also used the recombinant AEP ligase for in vitro cyclisation, ligation and site-specific labelling of a variety of non-cyclotide peptide targets.