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Progress towards the synthesis of piperazimycin A: synthesis of the non-proteogenic amino acids and elaboration into dipeptides

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ABSTRACT

This Letter describes the synthesis of the five non-proteogenic amino acids required for the total synthesis of piperazimycin A, and synthetic elaboration into multiple dipeptides. Importantly, this Letter details the first example of an elusive piperazic acid–piperazic acid coupling to form this key C5–C14 dipeptide.

Fenical and co-workers recently reported on the isolation of piperazimycin A **1** from the fermentation broth of a *Streptomyces* sp., cultivated from marine sediments near the island of Guam.[1] A cyclic hexadepsipeptide, **1** is composed of rare amino acids such as hydroxyacetic acid (HAA), α-methylserine, a novel (*S*)-2-amino-8-methyl-4,6-nonadecadienoic acid (AMNA), two γ-hydroxypiperazic acids ((*S,S*)-γOHPip and (*R,R*)-γOHPip and one γ-chloropiperazic acid (*R,S*)-γClPip (Fig. 1).[1] Piperazimycin A (**1**) proved to be a potent cancer cell cytotoxin which exhibited in vitro cytotoxicity towards multiple tumour cell lines with a mean GI_{50} of 100 nM.[1] Based on its novel molecular architecture, the diversity of non-proteogenic amino acid building blocks and its potent cytotoxicity, we embarked on a total synthesis campaign targeting piperazimycin A (**1**) in sufficient quantities to elucidate and pursue chemical biology studies.[2] In light of a recent total synthesis of piperazimycin A by Ma and co-workers (they coupled acyclic peptides and then cyclized to form the piperazic acids),[3] we describe here our progress towards piperazimycin A via fundamentally different synthetic approaches for the synthesis of both individual amino acids and dipeptides as well as the first example of a successful piperazic acid-piperazic acid coupling.

In order to complete a total synthesis of **1**, we first had to synthesize the requisite non-proteogenic amino acids **2**–**6** (Fig. 2). In a recent Letter, we described the synthesis of two γ-hydroxypiperazic acids, (*R,R*)-γOHPip **9** and (*S,S*)-γOHPip **10** and one γ-chloropiperazic acid (*R,S*)-γClPip **11** starting from either (*R*)- or (*S*)-4-chloro-3-hydroxybutanoic acid ethyl ester, **7** and **8**, respectively, in 8–9 steps via a key asymmetric Strecker reaction (Scheme 1).[2] For our current effort, we followed this route, but prepared Teoc derivatives **12**–**14** in place of the aforementioned Alloc congeners **9**–**11**. Overall yields for the eight step synthesis of **12** and **13** averaged 65%, and **14** was obtained in nine steps and 22% overall yield.

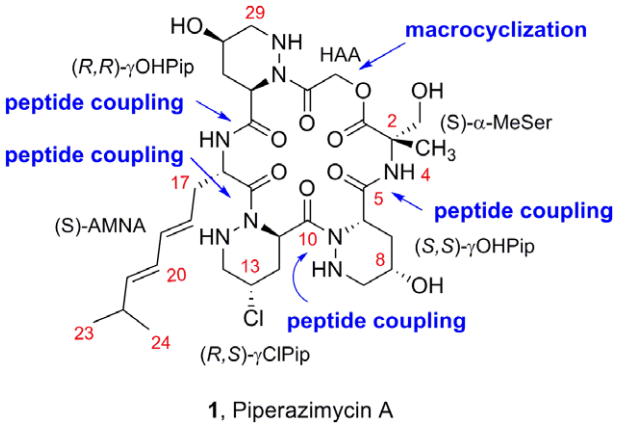

Figure 1. Structure of Piperazimycin A (**1**) and retrosynthetic plan.

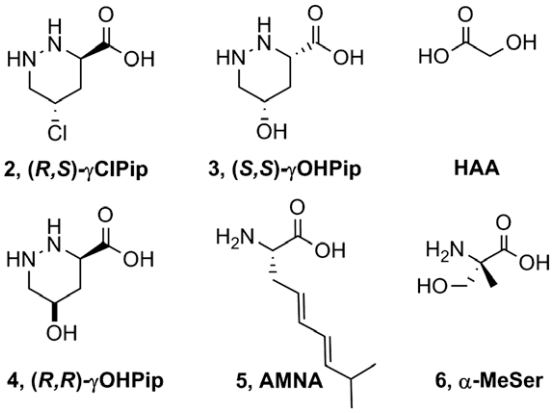

Figure 2. Non-proteogenic amino acids target molecules **2**–**6**.

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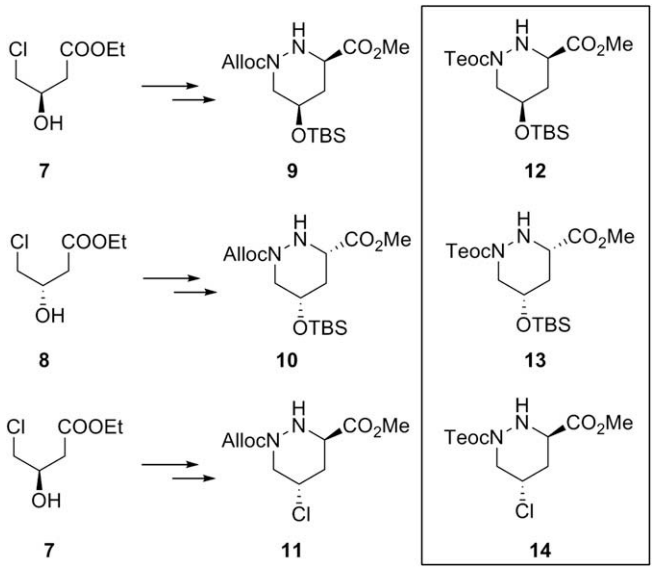


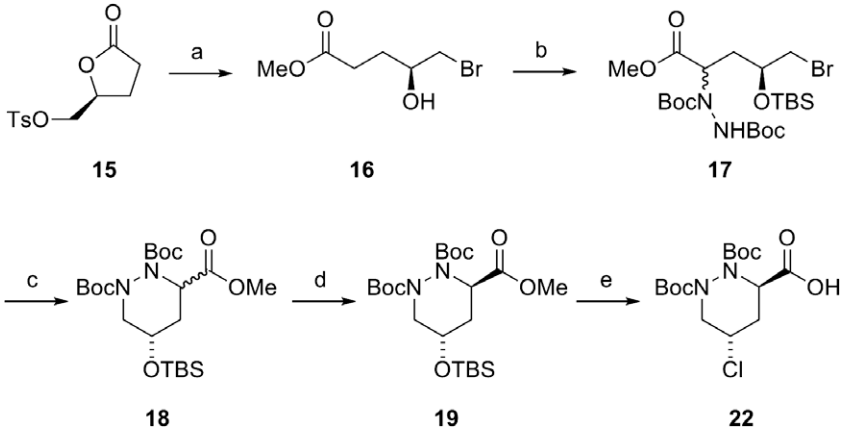
Scheme 1. Synthesis of N-protected, γ-substituted piperazic acids **9**–**14**.

From our experience thus far with piperazic acids, we also required a *bis*-Boc congener **22** to explore amide coupling conditions en route to a total synthesis of **1**. To this end, we followed a variation of Danishevsky's published route (Scheme 2).[4–6] Starting with the commercial (*S*)-lactone **15**, opening with methoxide, followed by conversion of the primary hydroxyl to the bromide affords **16** in 45% yield for the two steps. TBS protection of the secondary hydroxyl, followed by enolate formation and trapping with di-*tert*-butyl azodicarboxylate (DBAD) provides **17**, in 56% yield for the two steps. Deprotonation with NaH and cyclization delivers a 1:1 diastereomeric mixture of piperazic esters **18**. Careful column chromatography delivers (*R*,*S*)-γOTBSPip **19** in 40% yield. Removal of the TBS group, application of the Hale protocol[7] to install the γ-chloro functionality and hydrolysis provided the target **22** in 45% yield for the three steps. Overall, the eight-step sequence proceeded in 4.6% yield on multi-gram scales.

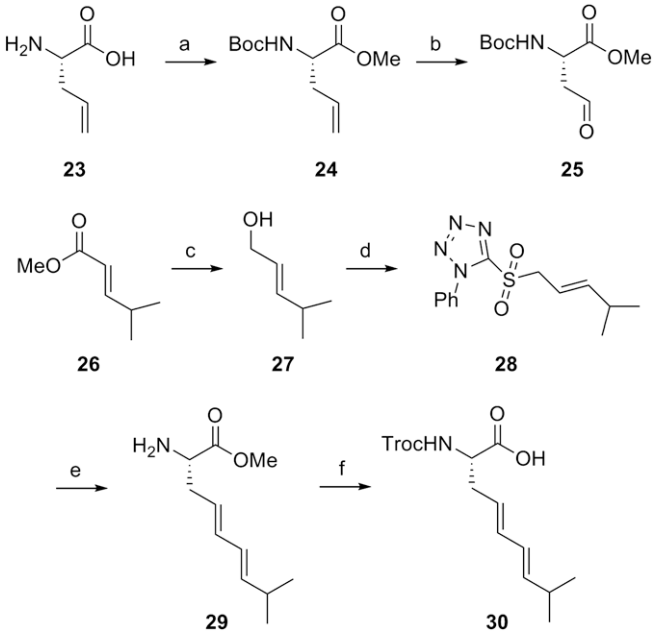
With all the requisite γ-substituted piperazic acids **12**–**14** and **22** in hand, attention now focused on preparing the unnatural AMNA, **5** (Scheme 3). Beginning with a commercial (*S*)-allyl glycine derivative **23**, Boc protection and esterification affords **24** in 96% yield. Ozonolysis delivers aldehyde **25** in 53% yield, and delivers one component for the envisioned Julia–Kocienski olefination[8–10] to provide the (*E*,*E*)-stereochemistry in **5**. The second component was derived from the commercially available (*E*)-methyl-4-methylpent-2-enoate **26**. Reduction with DIBALH provides, after distillation, allylic alcohol **27** in 75% yield. Deprotonation with *n*-BuLi, conversion to the tosylate and displacement of the allylic tosylate with the thiotetrazole and oxidation affords **28** in 53% yield for the four steps. The Julia–Kocienski olefination proceeds by deprotonation of **28** with KHMDS in DMF/HMPA to provide an 80:20 mixture of *E*/*Z* isomers.[8–10] Exposure to iodine and UV light isomerizes the diene to >95:5 *E*/*Z*. Deprotection of the Boc group with HCl delivers the bench stable AMNA congener, **29** in 70% yield for the three steps. **29** was also protected with the Troc group and the ester hydrolyzed to afford congener **30**.

Finally, we prepared the protected forms of α-methyl serine amino acid **6** for subsequent coupling (Scheme 4). Commercial (*S*)-2-amino-3-hydroxy-2-methylpropanoic acid **6** was Boc protected, followed by protection of the primary hydroxyl as a TBDPS ether to deliver **31** in 98% for the two steps. Compound **31** was then further elaborated with the HAA moiety to provide **32** to complete the northeastern fragment of piperazimycin A **1**.

With all the non-proteogenic amino acid components prepared, effort focused on construction of key dipeptides en route to a total synthesis of **1**. As described by Ma,[3] the peptide couplings were not trivial and each had to be optimized independently surveying dozens of coupling reagents, additives, solvents and alternative protecting groups on the various amino acid congeners of **2**–**6**. In the recently reported total synthesis of **1** by Ma,[3] they state that they were unable to affect the never before described piperazic acid-piperazic acid coupling between suitable congeners of **13** and **14**. Thus in their work, they followed the Danishefsky approach,[4–6] coupling acyclic amino acids and then cyclizing to form the piperazic acids as shown in Scheme 2. In our hands, we also were unable to couple analogues of **13** and **14**; however, protecting groups proved to be the key for this difficult transformation. Thus, the bis-Boc γ-ClPip **22** was successfully coupled to **13** in 62% yield employing freshly made tetramethyl chloroformamidinium hexafluorophosphate (TCFH), to form the acid chloride in situ, and provide **33**, and the first example of a piperazic acid-piperazic acid coupling (Scheme 5). The Boc groups were then removed with TFA and subsequent Teoc protection provided **34**, albeit in low yield (12% for the two steps).[11] Thus, the southern C5–C14 piperazic acid-piperazic acid fragment was prepared. However, we have



Scheme 2. Reagents and conditions: (a) (i) NaOMe, MeOH, (ii) LiBr, THF, HOAc, 45% for the two steps; (b) (i) TBSOTf, Imid, DMF, (ii) NaHMDS, THF, DBAD, 56% for the two steps; (c) NaH, DMF; (d) column chromatography, 40% over two (c and d) steps; (e) (i) 1 M TBAF, THF, 0 °C, (ii) NCS, PPh_3, DCM, (iii) 2 M LiOH, THF, 0 °C to rt, 45% for the three steps.



Scheme 3. Reagents and conditions: (a) (i) Boc_2O, TEA:DCM, (ii) MeI, TEA:DCM, 96% for two steps; (b) (i) O_3, DCM, −78 °C, (ii) DMS, 53%; (c) 2.2 equiv DIBALH, Et_2O, −78 °C, distillation, 75%; (d) (i) *n*-BuLi, 0 °C, (ii) TosCl, (iii) sodium 1-phenyl-1*H*-tetrazole-5-thiolate, 72% for three steps; (e) molybdenum, H_2O_2, EtOH, 98%; (f) (i) KHMDS, HMPA, DMF, (ii) **25**, (iii) I_2, UV light, Et_2O, HCl (g), 70% for three steps; (g) (i) TrocCl, DCM, TEA, (ii) 2 M LiOH, THF, 0 °C to rt, 90% for the two steps.

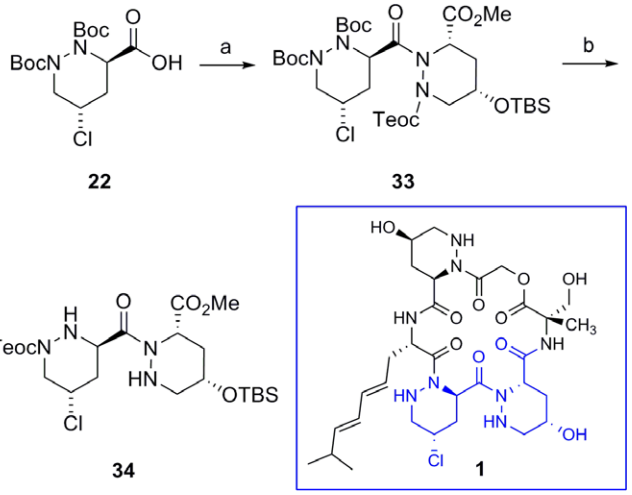
not been able to identify any conditions or protecting groups that allow **34** to be further elaborated.

To construct the eastern half of **1**, the Boc group of **32** was chemoselectively removed with HCl(g) in EtOAc to deliver **35**, which was then coupled to **36**, a hydrolyzed congener of **13**, under the standard HATU coupling conditions to deliver **37** in 26% yield for the two steps (Scheme 6).

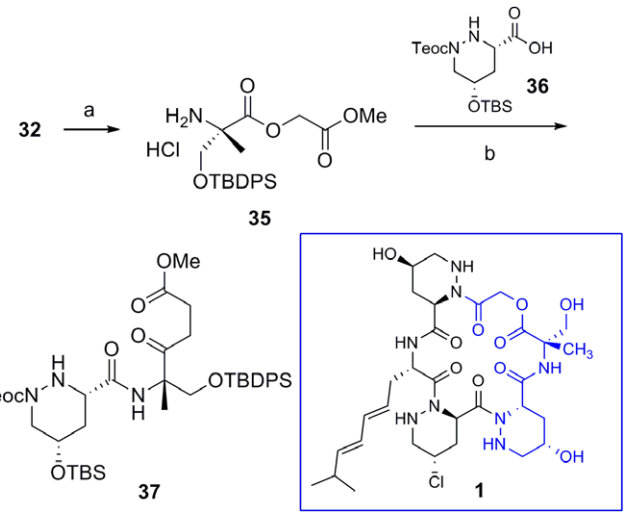
In parallel, the Troc-protected AMNA **30** was coupled to **14** under TCFH conditions (all others failed entirely) to deliver this dipeptide **38** in 49% yield. Hydrolysis generated **39** in a quantitative yield (Scheme 7).

The northwestern portion of **1** was also prepared (Scheme 8). γOHPip **12** was coupled to a PMB protected HAA congener **40** to provide **41** in 87% yield. Hydrolysis, subsequent HATU-mediated coupling with AMNA congener **29** and a second hydrolysis led to dipeptide **42** in 60% yield for the three steps. However, our most optimal coupling conditions thus far has proven ineffective to further elaborate any of the dipeptide fragments **34**, **37**, **39** or **42**. In order to complete the total synthesis of **1**, we will need to evaluate a more diverse array of peptide coupling strategies in combination with a diverse range of nitrogen-protecting groups on key peptide and dipeptide fragments.

In summary, we have synthesized all five of the non-proteogenic amino acids found in piperazimycin A **1**, and synthesized four advanced dipeptides (two with the HAA motif attached).

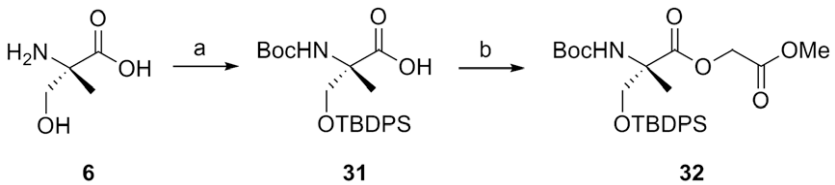


Scheme 5. Reagents and conditions: (a) **13**, TCFH, DCM, collidine, 0 °C to rt, 62%; (b) (i) TFA, DCM, 0 °C to rt, (ii) TeocCl, DMF, DIEA, 12% for two steps.

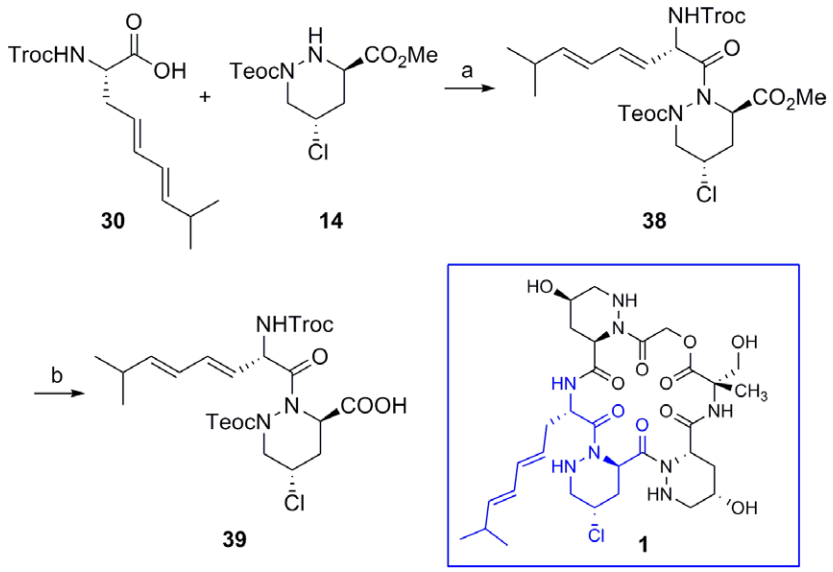


Scheme 6. Reagents and conditions: (a) HCl(g), EtOAc, 0 °C to rt, 100%; (b) (i) HATU, HOAt, DCM, collidine, 26%.

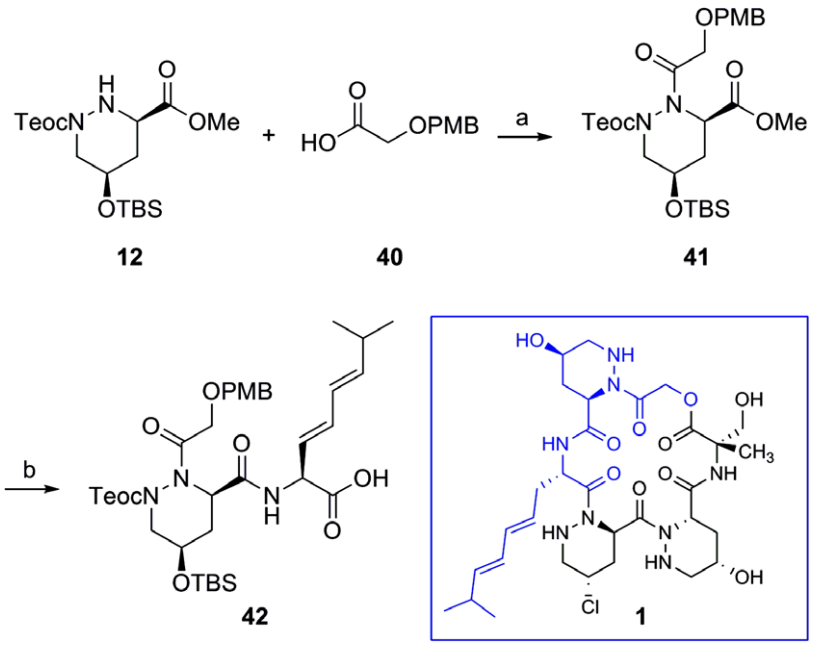
Importantly, we report on the first successful piperazic acid-piperazic acid coupling, mediated by TCFH, to synthesize the southern (*R,S*)-γClPip-(*S,S*)-γOHPip dipeptide **34**. We report our progress towards **1** due to the recent total synthesis reported by Ma and coworkers; moreover, our routes are fundamentally different for the synthesis of the individual non-proteogenic amino acids **2**–**6** found in piperazimycin A, and our approach to the dipeptide synthesis. Further refinements are underway towards a total synthesis of **1** and the related piperazimycin B, and will be reported in due course.



Scheme 4. Reagents and conditions: (a) (i) Boc_2O, TEA/DCM, (ii) TBDPSCl, imidazole, DMF, 98% for two steps; (b) NaH, Bromoacetic acid methyl ester 80%.



Scheme 7. Reagents and conditions: (a) TCFH, DCM, collidine, 0 °C to rt, 49%; (b) 2 M LiOH, THF, 0 °C to rt, quantitative.



Scheme 8. Reagents and conditions: (a) TCFH, DCM, collidine, 0 °C to rt, 87%; (b) (i) 2 M LiOH, THF, 0 °C to rt, 99%, (ii) HATU, HOAt, collidine, DCM, 62%, (iii) 2 M LiOH, THF, 0 °C to rt, 99%.

Acknowledgements

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11. *Experimental for the synthesis of* **33**: A flame-dried 50 ml flask outfitted with a stirbar was charged with acid **22** (160 mg, 0.439 mmol), and amine **13** (184 mg, 0.439 mmol) was vacuum purged 3x with argon. DCM (10 ml) followed by collidine (175 μl, 1.32 mmol) was added and the reaction was stirred until homogeneous. Freshly made TCFH (246 mg, 0.878 mmol) was added all at once and the reaction was stirred overnight. Once determined complete by LC/MS and TLC the reaction was quenched with H_2O and $NaHCO_3$ and the reaction mixture was extracted with DCM 3x. The organic layer was dried with $MgSO_4$, filtered and concentrated to afford the crude material which was purified by silica gel chromatography (EtOAc/Hex 1:3) to afford pure **33** in 62% yield (207 mg, 0.272 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.15 (m, 1H), 5.10 (m, 1H), 4.27 (m, 1H), 4.10 (m, 2H), 4.00 (m, 1H), 3.67 (s, 3H), 2.92 (m, 1H), 2.50 (m, 1H), 2.22 (m, 1H), 1.78 (m, 2H), 1.60 (m, 2H), 1.59 (s, 3H), 1.44 (s, 3H), 1.42 (s, 18H), 1.30 (m, 2H), 1.06 (m, 1H), 0.90 (s, 9H), 0.05 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 155.8, 153.2, 81.8, 64.9, 63.5, 51.9, 49.6, 48.8, 34.5, 31.7, 30.3, 29.7, 28.3, 26.9, 25.7, 25.3, 22.6, 20.7, 18.3, 14.1, −1.6, −5.1. HRMS (Q-ToF): *m*/*z* calcd for $C_{33}H_{61}N_4O_{10}NaSi_2Cl$ [M+Na] 787.3528, found 787.3512.