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Communication

The Synthesis of a Cubane-Substituted Dipeptide

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Amino acids and peptides bearing cyclic hydrocarbon side-chains are of interest in the development of a wide range of bioactive molecules. The preparation of an amino acid and a dipeptide derivative bearing an unfunctionalised cubane substituent is described. Attempts to prepare a cubylalanine derivative via the corresponding dehydroalanine were unsuccessful due to the high sensitivity of this vinyl cubane compound. Conversely, the addition of cubyllithium to a (R_S)-glyoxylate sulfinimine led to an effective synthesis of a cubylglycine derivative and a cubane-substituted dipeptide in diastereomerically pure form.

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Non-natural amino acids continue to attract attention as analogues of bioactive amino acids and for the incorporation into peptides for the purpose of developing metabolically stable, bioavailable peptides and peptidomimetics. Amino acids bearing cyclic hydrocarbon side-chains in particular have been pursued as analogues of glutamate and hydrophobic amino acids such as isoleucine. An interesting example of a peptide derivative bearing cyclic hydrocarbon side-chains is SEN304 (**1**, Fig. 1) which has been developed as a potential treatment for Alzheimer's disease.[1]

Conformationally rigid cyclic α-amino acids are of particular interest and are the subject of a detailed review by Komarov et al. published in 2004.[2] There are very few examples of the incorporation of such amino acids into peptides; cyclopropyl- and cyclobutyl-substituted amino acids appear to be the most common. The synthesis of amino acids with β-quaternary centres, however, can be challenging.[3] Adamantyl-substituted peptides are an intriguing class of bioactive molecules as they apparently benefit from an enhanced ability to penetrate biological membranes. Peptides bearing adamantyl side-chains have been investigated for their anti-tumour[4] and antimicrobial activities.[5] Cubane, structurally similar to adamantane, is a unique spherical saturated hydrocarbon molecule with eight methine units (C-H) arranged at the corners of a cube. Cubane possesses octahedral symmetry (O_h), is highly strained (strain energy of 695 kJ mol^{-1})[6] but stable, and has a density of 1.29 g cm^{-3}. To date, only one cubyl amino acid has been described, 4-carboxyl-cubylglycine (**2**, Fig. 2); this compound has been investigated for its neuroprotective properties.[7] To the best of our knowledge, there have been no reports of peptides bearing cubyl side-chains and there have been no cubane-substituted amino acids described where the cubane moiety is unfunctionalised. Amino acids bearing unfunctionalised cubane substituents are likely to be more appropriate mimics of hydrophobic amino acids such as leucine, isoleucine, and phenylalanine. Herein we report the first synthesis of an amino acid derivative bearing an unfunctionalised cubane side-chain and the first synthesis of a dipeptide derivative bearing a cubane side chain.

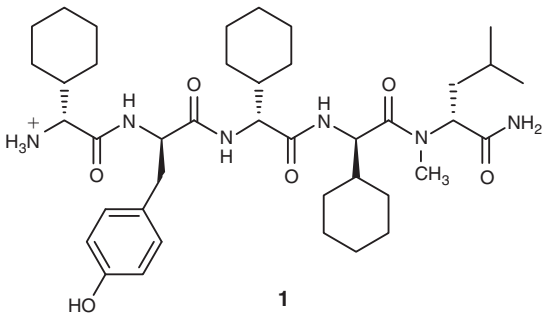

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Fig. 1. The structure of SEN304, a mimic of the peptide sequence LVFFL, which has been investigated as a potential treatment for Alzheimer's disease.[1]

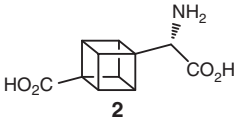

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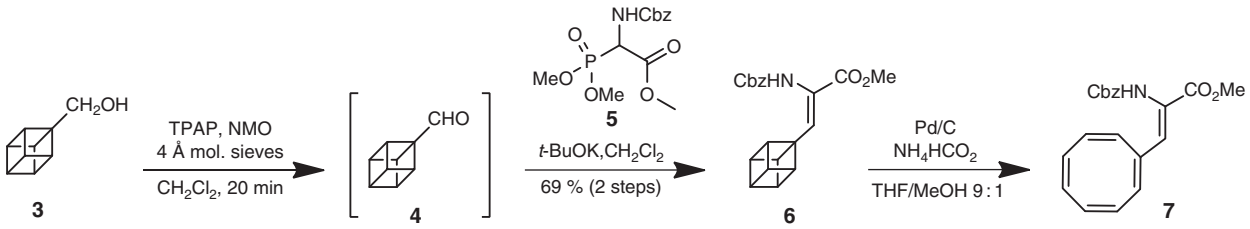
Fig. 2. The structure of a carboxyl-substituted cubylglycine investigated for its neuroprotective properties.[6]

Our first approach to an unfunctionalised cubyl amino acid employed a Horner-Wadsworth-Emmons reaction to install an

α,β-dehydroamino acid functionality. It was envisioned that asymmetric hydrogenation could be used to generate the desired cubylalanine derivative in enantiomerically enriched form. In order to obtain the required aldehyde precursor, cubylmethanol **3** (Scheme 1) was prepared from dimethyl 1,4-cubanedicarboxylate[8] in four steps via published procedures,[9] then cleanly oxidized to the cubane carbaldehyde **4** using tetrapropylammonium perruthenate catalyst (TPAP) and *N*-methyl morpholine-*N*-oxide (NMO). The resulting aldehyde **4** was found to be quite unstable, especially in concentrated form, and so was used in the next step without purification. The Horner-Wadsworth-Emmons reaction with glycine phosphonate **5** and potassium *t*-butoxide afforded the protected cubyldehydroalanine **6** in 69 % overall yield over two steps. Analysis of the product by ^{1}H and ^{13}C NMR spectroscopy indicated that only one geometrical isomer was present and the alkene was shown to have *Z*-geometry by X-ray crystallography (Fig. 3).

Cubane is known to undergo rearrangement reactions in the presence of several transition metals including rhodium (I), silver (I), and palladium (II),[10] so initial attempts to reduce the double bond present in **6** involved the use of $NaBH_4$, $CoCl_2/NaBH_4$,[11] and diimide.[12] In all of these cases only starting material was recovered. When the dehydroalanine derivative was subjected to hydrogenation using Pd/C and ammonium formate, rapid formation of the rearranged cyclooctatetraene derivative **7** was observed (Scheme 2 – the cyclooctatetraene derivative is assumed to have *Z*-configuration). The ease with which **6** undergoes rearrangement may relate to the slightly polarised nature of the vinyl substituent, which is thought to encourage such reactions in substituted cubanes.[13,14] An alternative is ruthenium catalysed hydrogenation, but for this to work efficiently a free pendant carboxylate or *N*-acyl functionality is required for coordination to the ruthenium.[15] The cubyldehydroalanine derivative **6** was hence saponified to provide the free carboxylate and the reaction mixture carefully neutralised. This process led, however, to complete decomposition, again demonstrating the instability of this type of vinyl cubane derivative.

Given the difficulties experienced with the cubane dehydroalanine system, we turned to the synthesis of a suitably protected cubylglycine derivative. Accordingly, iodocubane **8,** which was prepared in three steps from dimethyl



Scheme 1. Synthesis of protected cubyldehydroalanine **6** and attempted hydrogenation.

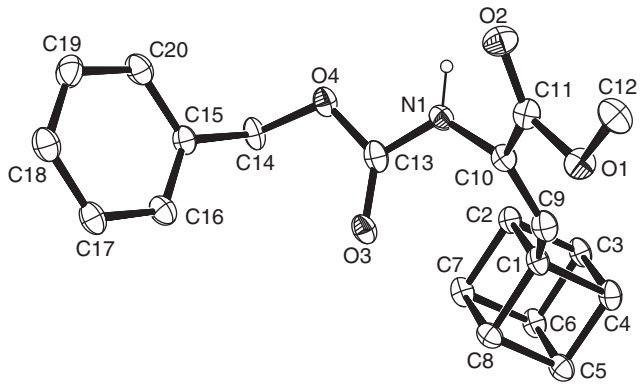
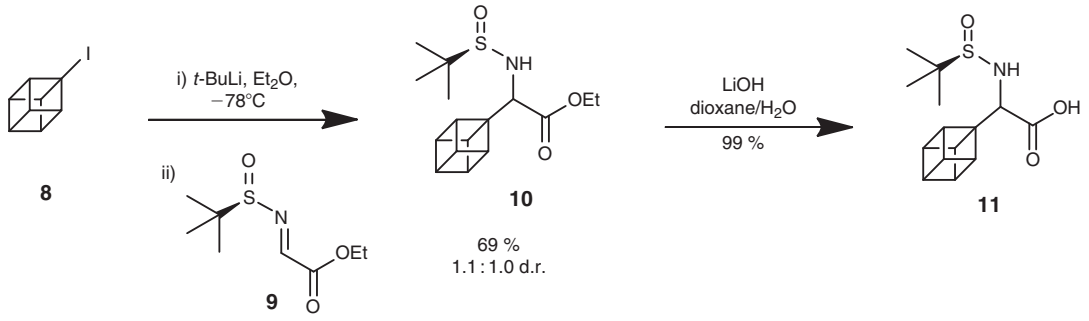


Fig. 3. View of **6**, with ellipsoids at 20 % probability, showing the alkene to have *Z*-geometry.



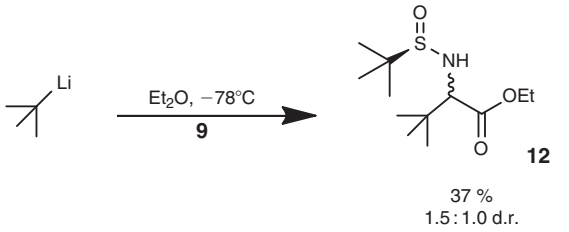
Scheme 2. Synthesis of cubylglycine derivative **11**.

1,4-cubanedicarboxylate,[16] was lithiated with *tert*-butyl lithium then treated with the (R_S)-glyoxylate sulfinimine **9**[17] to afford the corresponding amino ester **10** in good yield (Scheme 2). The nucleophilic addition to the chiral sulfinimine showed low diastereoselectivity (1.1 : 1.0). While the diastereoselective addition of organometallic nucleophiles into chiral sulfinimines is commonly reported,[18] examples of additions of tertiary alkyl lithium nucleophiles into these systems are rare. To investigate whether the low diastereoselectivity we observed is unique to the cubyl system or if it is a general characteristic of the addition of tertiary organolithium nucleophiles to **9**, we investigated the addition of *tert*-butyllithium to the sulfinimine. Interestingly, this reaction also proceeded in poor diastereoselectivity (1.5 : 1.0, Scheme 3). In selected cases, when the addition of nucleophiles into chiral sulfinimines has resulted in low diastereoselectivity, Ellman's group has been able to improve the diastereoselectivity by the use of Lewis acids such as $BF_3{\cdot}Et_2O$ or trimethylaluminium.[19] In this instance, however, the use of either $BF_3{\cdot}Et_2O$ or trimethylaluminium additives in the addition reaction of cubyllithium to **9** led to complex reaction mixtures.

Hydrolysis of cubylglycine ethyl ester **10** with lithium hydroxide afforded the corresponding amino acid **11** in near quantitative yield. Previously it has been noted that *t*-butylsulfinamide amino acid diastereomers can have significantly different solubility, and this trait can be used to diastereomerically enrich a diastereomeric mixture.[20] Accordingly, the carboxylic acid **11** could be diastereomerically enriched to >9 : 1 d.r. by trituration with acetone. With a method for resolving the diastereomers in hand, we then sought to confirm the absolute stereochemistry of the major diastereomer of **11** following enrichment. An enriched mixture of the cubylglycine derivative **11** was hence converted to the corresponding phenylglycine dipeptide **13** by standard HBTU (*O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) coupling conditions with (*R*)-phenylglycine methyl ester (Scheme 4). By examination of the ^{1}H NMR spectra of the two diastereomers of **13**, we were able to use Yabuuchi and Kusumi's model[21,22] to assign the major diastereomer of **13** as (R_s,*S*,*R*) since, relative to the minor isomer, the ^{1}H NMR peaks corresponding to the cubane moiety appeared upfield and the peaks corresponding to the *t*-butyl moiety appeared downfield, as a result of the ring current effect of the phenyl moiety (see Fig. 4 and Supplementary Material). By implication, the minor diastereomer was assigned to have (R_s,*S*,*R*) configuration.

The generality of **11** to serve as a precursor for cubane-substituted peptides was then demonstrated through the preparation of the L-leucine dipeptide **14** (Scheme 5) which was obtained in diastereomerically pure (R_s,*S*,*R*) form via chromatography over silica.

At the present time a suitable method for unmasking of the *N*-terminal nitrogen has not been identified. Treatment of **14** with standard acidic conditions[23] gave no reaction, whereas increased reaction time and/or the application of heat led to decomposition of the peptide. In order to obtain more information about potential methods for the cleavage of the *N*-sulfinamide moiety we investigated the deprotection of the cubylglycine ester **10**. No reaction was observed when the standard deprotection conditions (0.4 M HCl in methanol) were applied to this substrate, and increased acid concentration and excess (20 eq) or extended reaction times (4 days) gave the same result. Hydrochloric acid promoted deprotection of the *t*-butylsulfinamide group is reliant on nucleophilic attack of chloride to displace the sulfinyl moiety.[24] It is reasonable to expect that the bulky nature of the cubane substituent might prevent this nucleophilic displacement from occurring. It was envisaged that oxidation of the *t*-butylsulfinamide group to the acid labile *t*-butylsulfonamide (BUS-derivative), might facilitate cleavage of the S-N bond and so the sulfinamide **10** was cleanly oxidized to the corresponding sulfonamide **15** using standard *m*-chloroperbenzoic acid (*m*-CPBA) conditions (Scheme 6).[25] Attempts were then made to cleave the *t*-butylsulfonamide of **15** involving the use of triflic acid/anisole, triflic acid/trifluoroacetic acid, or $AlCl_3$/anisole,[26,27] all of which led to recovered starting material. It is clear that



Scheme 3. The addition of *tert*-butyl lithium to the glyoxylate sulfinimine **9**.

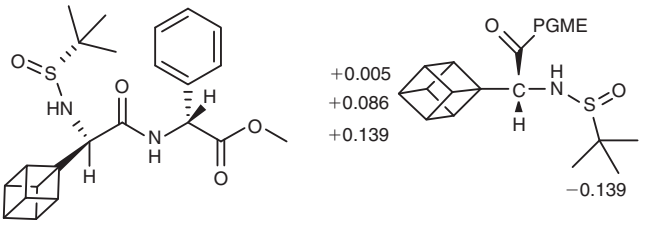
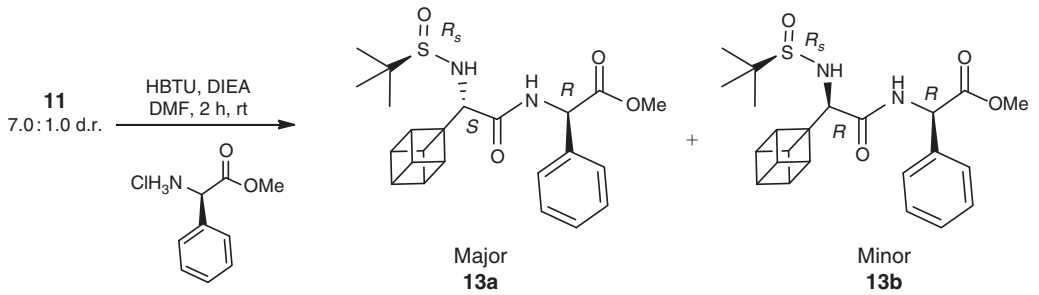
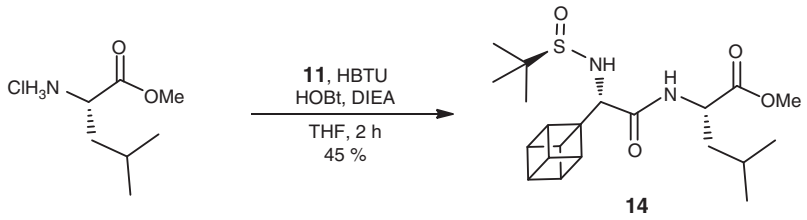


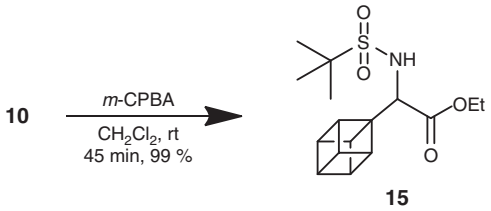
Fig. 4. Diastereomeric δ NMR values and predicted conformation of cubylglycine-*R*-phenylglycine dipeptide **13a** according to Yabuuchi and Kusumi's model.[21,22] PGME: phenylglycine methyl ester.



Scheme 4. Synthesis of cubylglycine-phenylglycine dipeptide derivative **13**. DIEA: diisopropylethylamine.



Scheme 5. Synthesis of R_s,*S*,*S*-cubylglycine-leucine dipeptide **14**. HOBt: 1-hydroxybenzotriazole.



Scheme 6. Conversion of the *N*-sulfinamide derivative of cubylglycine ester **10** to the *N*-sulfonamide **15**.

sulfinamide and sulfonamide derivatives of cubylglycine are remarkably resistant to cleavage.

In summary, we have synthesised the first cubyl amino acid derivative bearing an unfunctionalised cubane moiety **11** and prepared the first known example of a cubyl-substituted peptide **14**. The tolerance of cubylglycine derivatives to harsh reaction conditions such as concentrated Lewis and Bronsted acids has been shown, but cubyldehydroalanine derivatives were found to readily decompose or rearrange to the corresponding cyclooctatetraene derivatives in the presence of palladium or acid.

While the use of the enantiomerically pure glyoxylate sulfinimine **9** in the addition reaction of cubyllithium did not lead to a high diastereoselectivity, the presence of the chiral *t*-butylsulfinamide group in the product allowed for the simple enrichment of one of the diastereomers of the cubylglycine derivative **11**. Coupling of a diastereomerically enriched form of this acid with *R*-phenylglycine facilitated the determination of the configuration of the two diastereomers of **11** through the use of Yabuuchi and Kusumi's ^{1}H NMR method.

Supplementary Material

Experimental procedures and characterisation data for all new compounds as well as the cif file for **6** are available on the Journal's website.

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