Total Synthesis of Putative Structure of Asperipin-2a and Stereochemical Reassignment

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Supporting Information Placeholder

ABSTRACT: The total synthesis of the putative structure of asperipin-2a is described. The synthesis features ether cross-links between the phenolic oxygen of Tyr^6 and β position of Tyr^3 and the phenolic oxygen of Tyr^3 and the β position of Hpp^1 in the unique 17- and 14-membered bicyclic structure of asperipin-2a, respectively. The synthesized putative structure does not match the natural product and a stereochemical reassignment is postulated.

Asperipin-2a is a ribosomally synthesized and post-translationally modified peptide (RiPP) isolated from *Aspergillus flavus* (Figure 1).[1]

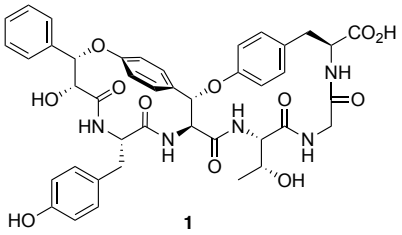


Figure 1. Proposed structure of asperipin-2a **1**.

The gene cluster encoding asperipin-2a was discovered through a search for fungal gene clusters related to those involved in the biosynthesis of the ustiloxins. Four genes encode the proteins responsible for biosynthesis of asperipin-2a: AprA, the precursor peptide; aprY, an oxidase; aprT, a transporter; and aprR, a reductase.[1-7] The two-dimensional structure of asperipin-2a was reported in 2016,[1] with the biosynthetic pathway and absolute configuration unveiled in 2019 (Figure 2).[7]

Asperipin-2a is a bicyclic hexapeptide derived from the sequence FYYTGY. The precursor peptide is first processed by KexB protease (encoded outside the asperipin gene cluster) then the fungi-specific DUF3328 oxidase, AprY, catalyzes formation of the two ether cross-links; between the phenolic oxygen of Tyr-6 and β-position of Tyr-3, and the phenolic oxygen of Tyr-3 and β-position of Phe-1. The Phe-1 residue is transformed into a 2-hydroxypropanoic acid (Hpp-1) residue through an oxidation–hydrolysis–reduction process. The unique structure of asperipin-2a, together with the lack of information regarding its biological role or activity and our interest in cross-linked peptide natural products,[8-10] prompted us to investigate a total synthesis of this compound. We previously reported a route to the C-terminal macrocycle of asperipin-2a.[11] Herein, we report the first total synthesis of the putative structure of asperipin-2a, and postulate a stereochemical reassignment of the Hpp residue on the N-terminal ring.

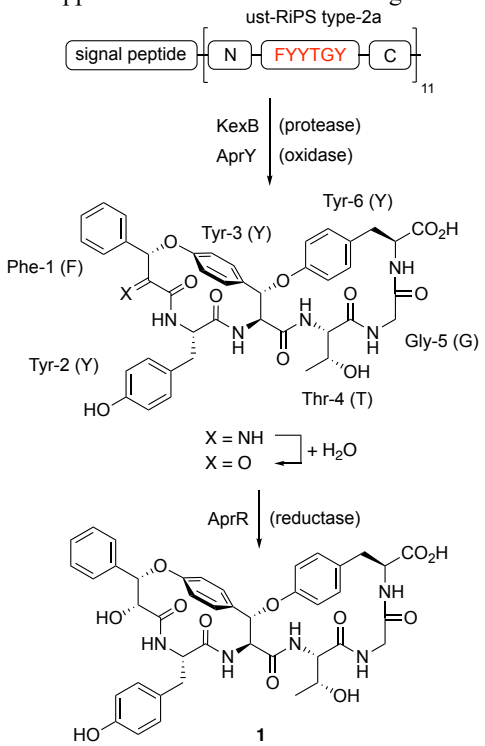
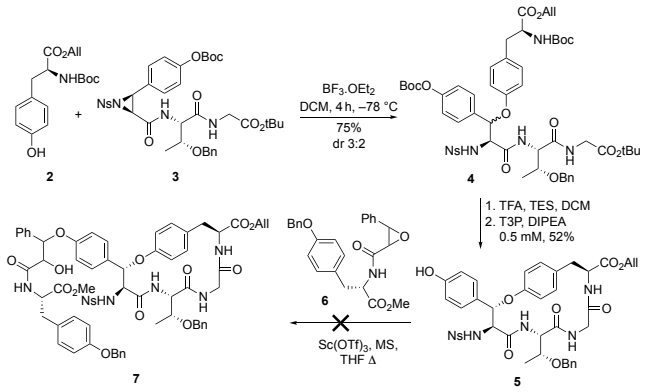


Figure 2. Biosynthesis of asperipin-2a.

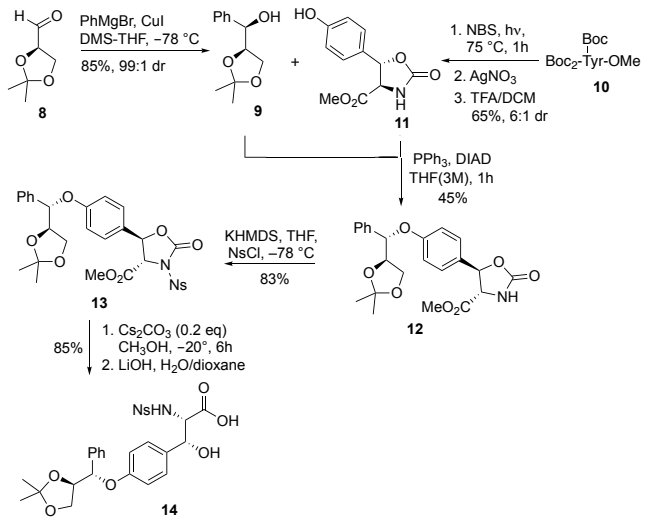
The main challenge in the total synthesis of asperipin-2a is the formation of the two alkyl–aryl ether linkages in the highly functionalized bicyclic system. A number of methods including epoxide ring opening[12] and Mitsunobu reactions[13,14] have been reported for the synthesis of benzyl–aryl ethers. We initially postulated an aziridine/epoxide ring-opening approach toward both ether linkages in asperipin-2a.[11] Modification of the tyrosine protecting group employed in our previously reported synthesis of the right hand ring furnished the phenol **5** (Scheme 1 and SI). However, all attempts at ring-opening of epoxide **6** with the phenolic group of **5** to generate the second aryl–benzyl ether linkage in **7** were unsuccessful.

Scheme 1. Attempted generation of second ether linkage through epoxide opening.



With the epoxide ring-opening approach not providing the desired results, we switched to a Mitsunobu approach. Accordingly, (*R*)-glyceraldehyde acetonide **8** was treated with phenylmagnesium bromide in the presence of CuI to give the alcohol **9** in 99:1 d.r.[15,16] Analysis of **9** by X-ray crystallography confirmed the (*R,R*)-configuration (Figure 3). The Mitsunobu reaction of alcohol **9** with tyrosine derivative **11** under sonication and at high concentration (3 M) successfully generated ether adduct **12**. Compound **12** was nosylated then the oxazolidinone and ester groups of **13** were hydrolyzed to afford the β-hydroxytyrosine derivative **14** (Scheme 2).

Scheme 2. Synthesis of β-hydroxytyrosine **12**.



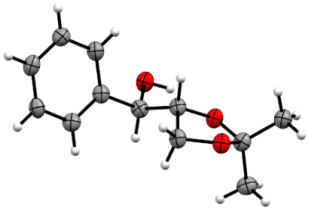
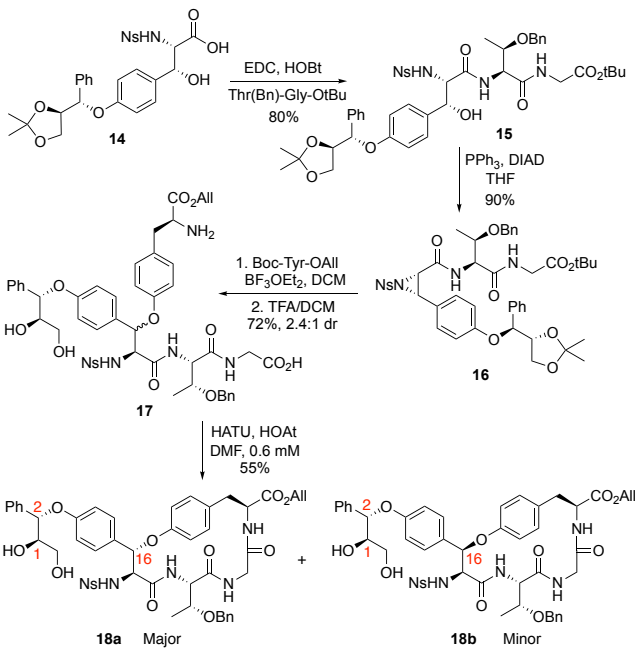


Figure 3. ORTEP diagram of **9** (ellipsoids at the 30% probability level).

The β-hydroxytyrosine **14** was coupled with Thr(Bn)-Gly-O*t*Bu to give the peptide **15** in 80% yield. Formation of the Tyr-3–Tyr-6 ether cross-link was then pursued using our aziridine strategy. The β-hydroxytyrosine moiety of peptide **15** was converted to the aziridine **16** in 90% yield as a single diastereomer. Treatment of the aziridine **16** with protected tyrosine Boc-Tyr-OAll **2** in the presence of BF_3•OEt_2, followed by deprotection of acetonide, *t*Bu and Boc groups, yielded ether-linked adduct **17** in 72% yield and 2.4:1 d.r. (Scheme 3). While the diastereoselectivity of the aziridine ring opening is moderate, it was an improvement over our model studies.[11]

Scheme 3. Synthesis of macrocycle **18**.



Macrocyclization of peptide **17** using HATU/HOAt afforded a diastereomeric mixture of **18** in 55% yield. The isomers were separated by HPLC and the minor diastereomer **18b** was successfully crystallized and analyzed by X-ray crystallography, which confirmed the 1*R*,2*S*,16*R*-stereochemistry (Figure 4, numbering according to that of asperipin-2a). The configuration of the central β-hydroxytyrosine moiety was in accordance with our previous study.[11] Thus, the major isomer **18a** possesses 1*R*,2*S*,16*S*-stereochemistry. At this stage of our synthesis, the absolute configuration of asperipin-2a was reported by Ye et al as 1*R*,2*S*,16*S*,[7] suggesting the 2*S* and 16*S* configurations of **18a** match those of the natural product. The configuration at the C1 secondary alcohol of **18a** is opposite of that predicted by Ye et al, requiring an inversion step later in the synthesis (Note; the *R*/*S*-notation at C1 is switched due to a difference in priorities

between the primary alcohol present in **18a** and the amide group in asperipin-2a **1**).

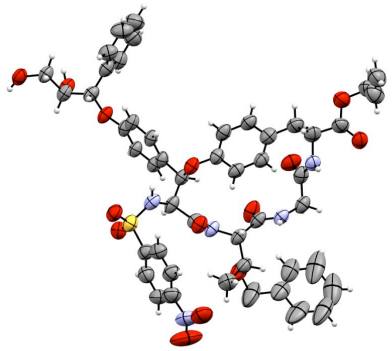
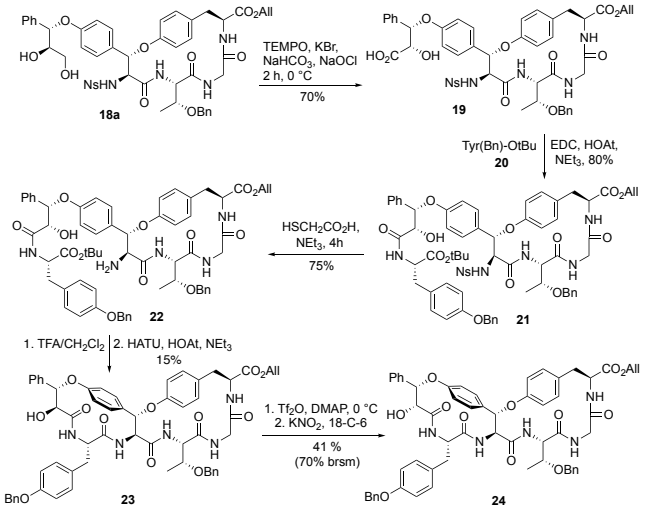


Figure 4. ORTEP diagram of **18b** (ellipsoids at the 30% probability level).

Next, selective oxidation of the primary alcohol of **18a**, in the presence of the secondary alcohol, was pursued. Treatment of **18a** with TEMPO and NaOCl was effective, generating carboxylic acid **19** in 70% yield (Scheme 4).[17] Coupling of **19** with Tyr(Bn)-O*t*Bu **20** afforded peptide **21** in 80% yield, then deprotection of the nosyl group and *tert*-butyl esters afforded **22** in 75% yield. Macrocyclization of **22** was effected using HATU and HOAt to provide the bicyclic compound **23** in 15% yield. The corresponding cyclodimer was isolated as the major product in 25% yield.

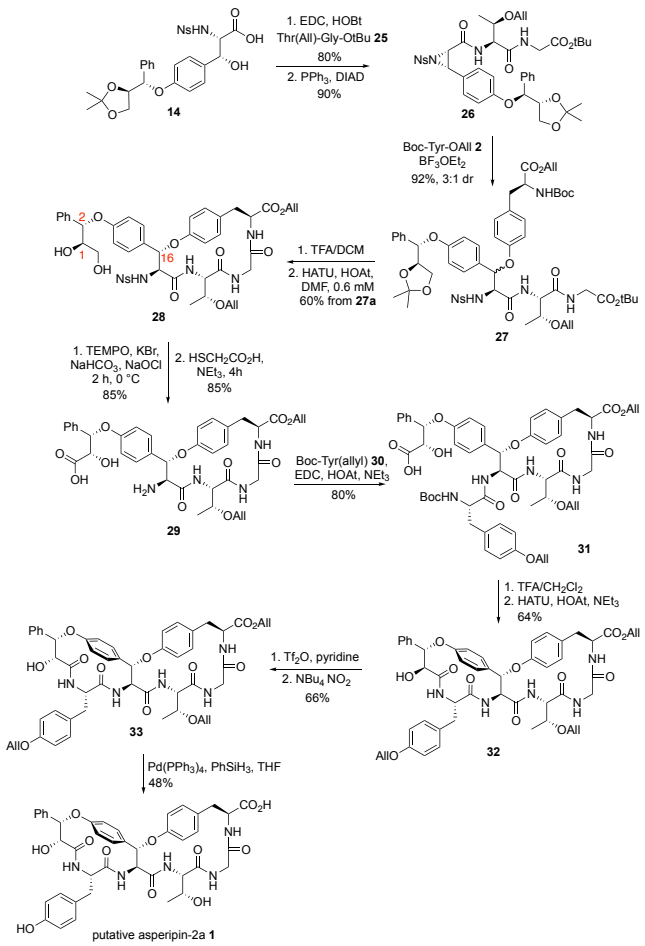
Scheme 4. Synthesis of protected asperipin **24**.



Despite the low yielding macrocyclization, with the bicyclic framework of asperipin-2a successfully constructed, conversion to asperipin-2a was pursued. Inversion of the secondary alcohol at C1 was required to generate the absolute configuration of asperipin deduced by Ye et al. Accordingly, a Lattrell-Dax reaction[18,19] was performed on **23** to provide protected asperipin-2a **24** in 70% yield. Unfortunately, at this stage all attempts to cleave the benzyl groups from **24** resulted in decomposition. We postulate that the ether linkages in **24** are not stable to hydrogenolysis conditions.

In order to access a total synthesis of asperipin-2a, a modified protecting group strategy was required. Accordingly, we employed a global allyl-protecting group strategy. Further, given the issue of cyclodimerization during formation of the second macrocycle, an alternative macrocyclization position was pursued. Allyl-protected threonine derivative **25** was first coupled to β-hydroxytyrosine **14**, and the resultant peptide was converted to the aziridine **26** in 90% yield (Scheme 5). Subsequent aziridine ring opening on **26** with Boc-Tyr-Oallyl **2** afforded ether-linked adduct in a slightly improved 3:1 dr. Following separation of the diastereomers, deprotection of the acetonide, *t*Bu and Boc groups of the major isomer using TFA yielded peptide **27**. Macrocyclization of **27** using HATU/HOAt afforded macrocycle **28** in 60% yield. Selective oxidation of the primary alcohol in the presence of the secondary alcohol was again effected by treatment with TEMPO and NaOCl to generate the corresponding carboxylic acid in 85% yield. Deprotection of the nosyl group afforded hydroxy acid **29** in 85% yield (Scheme 5).

Scheme 5. Synthesis of asperipin-2a **1**.



In order to investigate an improved route to the N-terminal ring, we decided to perform the macrocyclization at the Hpp-1–Tyr-2 amide bond, rather than the Tyr-2–Tyr-3 attempted previously. Accordingly, Boc-Tyr(allyl)-OH **30** was activated with EDC then coupled to the amine of **29** to afford peptide **31** in 80% yield. The Boc group was removed by treatment with TFA and subsequent macrocyclization using HATU and HOAt successfully provided the bicyclic peptide **32** in good yield (64%) with no evidence of cyclodimerization. Lattrell-Dax inversion of the configuration of the secondary alcohol at C1 of **32** was effected with tetrabutyl ammonium nitrite, which proceeded in better yield than using KNO_2, to afford the protected asperipin-2a **33**. Global deprotection of the three allyl groups was effected

by treatment with $Pd(PPh_3)_4/PhSiH_3$ to provide putative asperipin-2a **1** in 48% yield (Scheme 5).

Comparison of the ^1H NMR data of the natural product and the synthesized compound revealed significant differences, most notably for the signals corresponding to the hydrogen at C2. A sample of asperipin-2a natural product was kindly donated by Prof Oikawa. The H2 signal in ^1H NMR spectrum of natural asperipin-2a is observed as a broad singlet peak at δ 5.96 ppm, with the small coupling constant to H1 indicative of a dihedral angle H1–C1–C2–H2 of close to 90°. However, the corresponding signal of H2 in the synthesized compound appeared as a doublet at δ 5.58 ppm with coupling constant 9.0 Hz to H1 (Figure 5). Further, HPLC analysis of the natural and synthesized compounds indicated they are not identical (Figure 6).

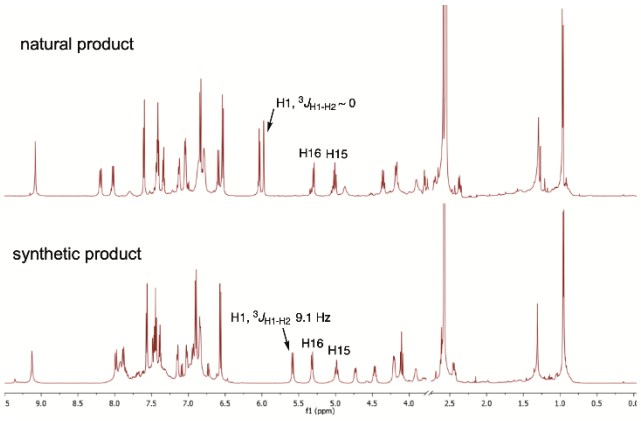


Figure 5. NMR comparison of natural and synthetic asperipin.

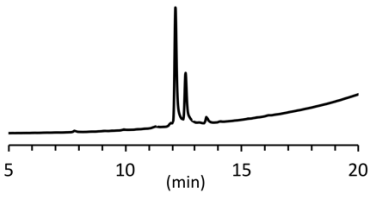


Figure 6. HPLC traces of co-injected natural and synthetic asperipin. Synthetic product retention time: 12.16 min. Natural product retention time: 12.61 min. Wavelength: 214 nm.

With the synthesized asperipin not matching the natural product we sought to determine the point of discrepancy. The configuration at the α-Cs of Hpp-1, Tyr-2, Tyr-3, Thr-4 and Tyr-6 was unambiguously established by Oikawa and co-workers through a hydrogenolysis–hydrolysis of the natural product and analysis of the constituent residues.[7] The amino acids were converted to their L-FDLA derivatives and analyzed by LC-MS. Chiral HPLC analysis of the underivatized hydrolysate confirmed the presence of (*R*)-3-phenyllactic acid, indicating the configuration at C1 (the α-C of Hpp-1) to be (*R*). Following this analysis, the remaining stereocentres to be determined are the β-positions of Hpp-1 and Tyr-3; C2 and C16. The (*S*)-configuration at C16 was confirmed by both NOE analysis (ref. 7) and X-ray crystallography (Figure 4 and ref. 11).

Thus, only the configuration at C2 remains ambiguous. Oikawa and coworkers deduced the relative stereochemistry at C1–C2 through *J*-value analysis, where the small coupling between H1 and H2 is indicative of a Gauche or near-perpendicular orientation. Oikawa and coworkers proposed a conformation as shown in Figure 7A, in which H1 and H2 are in a near-Gauche orientation, thus assigning the 2*S*-configuration at C2. However, the 1*R*,2*R*-configuration could also exist in a conformation that generates a small *J* value (Figure 7D). In each of these conformations (A and D) H1 and H2 are in a near-perpendicular orientation. The OAr and CONHR groups are in a near perpendicular orientation in 7A, and a near *syn* orientation in 7D: these groups are connected through the N-terminal ring, which could be accommodated with either configuration.

The stereochemistry of the synthetic compound, which is clearly not asperipin-2a, is established by X-ray analysis of **18a**, demonstrated to possess 1*R*,2*S*- (*erythro*-) absolute stereochemistry, and by analogy the same holds for intermediate **27**. Compound **27** must therefore be transformed to the 1*S*,2*S*- (*erythro*-) precursor **32** (due to change in CIP priorities upon oxidation of the primary alcohol to the acid). Latrell-Dax inversion at C1 of **32** must therefore generate the 1*R*,2*S*- (*threo*-) synthetic product **1** (Figure 7B).

Thus, with the synthetic and natural compounds being non-identical, and C2 of the natural product being the only ambiguous stereocentre, we propose that natural asperipin-2a must be the 1*R*,2*R*-isomer, with a conformation shown in Figure 7D.

Further evidence is provided by NMR analysis of precursor **32**, in which the H1 signal is observed as a doublet at δ 5.96 ppm with a small coupling constant of 3.2 Hz (Figure S126), similar to that observed in the natural product. This similarity suggests that **32** and natural asperipin-2a possesses the same relative stereochemistry at C1–C2. As **32** possesses 1*S*,2*S*- configuration, and natural asperipin-2a is 1*R*, this analysis supports the 1*R*,2*R*-assignment for natural asperipin-2a (Figure 7C/D).

This our revised assignment of the stereochemistry of asperipin-2a is that the natural product is 1*R*,2*R*-asperipin, and our synthetic compound is 1*R*,2*S*-asperipin-2a, or 2-*epi*-asperipin (Figure 8).

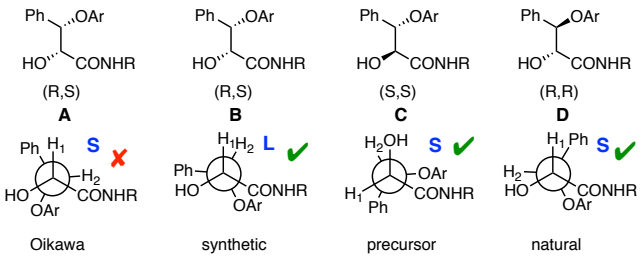


Figure 7. Newman projections of C1–C2 isomers of asperipin. S = small predicted J_{H1-H2}, L = large predicted J_{H1-H2}.

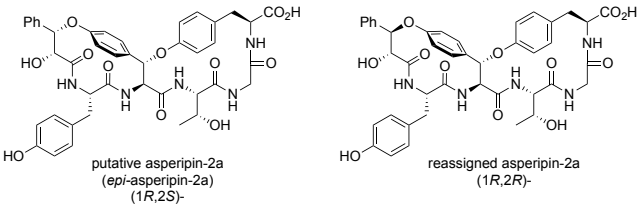


Figure 8. Reassigned stereochemical assignment of asperipin-2a.

In conclusion, we have completed the first total synthesis of the putative structure of the bicyclic peptide natural product asperipin-2a. The synthesized compound was not identical to the natural product, prompting reanalysis of the stereochemistry of asperipin-2a. We conclude that natural asperipin-2a possesses (1*R*,2*R*)-stereochemistry.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization (NMR, MS spectra, HLPC traces; PDF).

Accession Codes

CCDC 2019840 (**9**) and 2019841 (**18b**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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