JOC The Journal of Organic Chemistry

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Total Synthesis of Unguisin A

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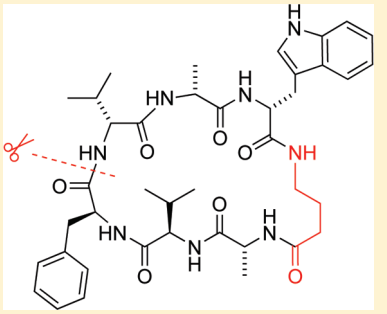
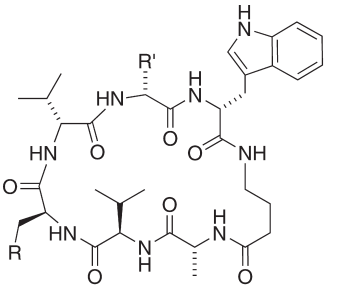
Supporting Information

ABSTRACT: The first synthesis of the γ-aminobutyric acid (GABA)-containing cyclic heptapeptide unguisin A is reported, confirming the structure of this natural product. Macrocyclization of a flexible GABA-containing linear precursor is found to proceed rapidly and in good yield.



Naturally occurring cyclic peptides have attracted considerable attention due to their diverse biological activity and significant therapeutic potential.[1] Cyclic peptides possess several pharmacokinetic advantages relative to their linear counterparts including greater metabolic stability, higher hydrophobicity, and increased conformational rigidity, and as such they are attractive scaffolds for drug development.[2] Natural cyclic peptides are also of intrinsic interest due to their fascinating structural variety, often featuring unusual amino acid components that can be created through posttranslational modification of a ribosomally assembled peptide[3] or by nonribosomal incorporation of nonproteinogenic amino acids.[4]

Unguisins A−C (**1**−**3**, Figure 1) are cyclic heptapeptides isolated from the marine fungus *Emericella unguis*.[5,6] Notable structural features of these molecules include a predominance of hydrophobic amino acid side chains, a high proportion of D-amino acids, and the highly unusual feature of a γ-aminobutyric acid (GABA) residue contained within the macrocycle. Several natural products containing β-hydroxy-γ-aminobutyric acid (GABOB) residues have previously been described (e.g., microsclerodermins A−E from *Theonella* sp. and *Microscleroderma* sp.[7]), and other natural products containing GABA residues within linear peptide segments have been identified (e.g., imacidins A−E from *Streptomyces olivaceus*[8] and some *N*-acylasparagylpolyamines from *Nephilengys borbonica*[9]); however, to the best of our knowledge, the unguisins (**1**−**3**) are the only natural products reported to date that contain a GABA residue within a cyclic peptide structure. This feature strongly suggests a nonribosomal biosynthetic origin, and this hypothesis is also supported by the results of precursor-directed biosynthesis experiments.[6] When *E. unguis* was cultured in the presence of excess L-leucine, a new metabolite (denoted unguisin D) was isolated in which a D-valine residue of unguisin B is replaced by L-leucine,[6] and this finding was attributed to the characteristically low specificity exhibited by some nonribosomal peptide synthase multienzymes.[10]



Unguisin A (**1**): R = Ph, R' = CH_3
Unguisin B (**2**): R = ^{i}Pr, R' = CH_3
Unguisin C (**3**): R = Ph, R' = CH_2OH

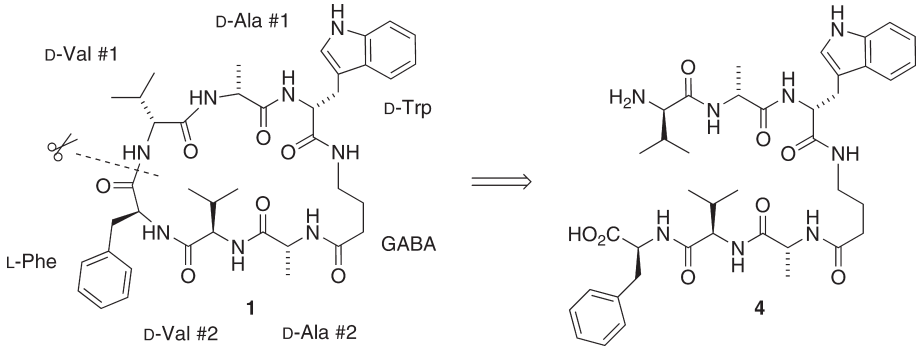
Figure 1. Structures of unguisins A−C.

Unguisins A and B are reported to possess moderate antibacterial activity against *Staphylococcus aureus*;[5] however, these effects have not been quantified, and full biological profiling is lacking. It has been speculated that the GABA components of **1**−**3** may impart some degree of conformational flexibility which may allow the macrocycles to adopt the required shape for optimal biological activity; however, this has not been explored through structure−activity relationship studies. Therefore, to further investigate this intriguing molecular scaffold, we undertook to perform, and report herein, a total synthesis of unguisin A (**1**).

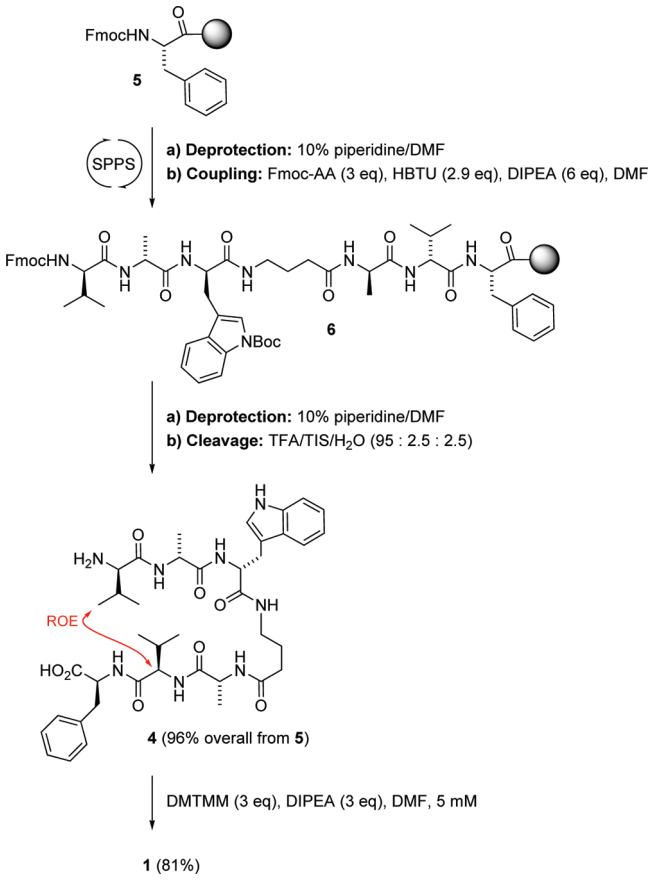
The retrosynthetic analysis of **1** (Scheme 1) involves disconnection of the macrocycle between the phenylalanine carboxyl group and the adjacent valine amino group. This disconnection site was chosen for two reasons. First, in the forward sense the synthesis would involve a macrocyclization reaction between

Received: April 20, 2011
Published: May 25, 2011

Scheme 1. Retrosynthetic Analysis of Unguisin A (1)



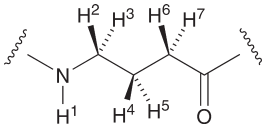
Scheme 2. Synthesis of Unguisin A (1)[a]



[a] Abbreviations: black sphere = Wang resin, 100−200 mesh, amino acid loading 0.65 mmol/g; DMF = *N*,*N*-dimethylformamide; HBTU = *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA = diisopropylethylamine; TFA = trifluoroacetic acid; TIS = triisopropylsilane; DMTMM = 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium tetrafluoroborate.

L- and D-amino acid residues, which has previously been identified as beneficial in reducing epimerization and cyclooligomerization reactions.[11] Second, the chosen disconnection site would position the GABA residue in the center of the linear precursor **4**, and this flexible central component could potentially facilitate the folded conformation required for efficient macrocyclization.

Table 1. Selected Coupling Constants (Hz) from the GABA Residue of 1[a,b]



	H^2	H^3	H^4	H^5	H^6	H^7
H^1	6.2	4.8	0.0	0.0	0.0	0.0
H^2		14.0	7.0	6.0	0.0	0.0
H^3			7.0	6.0	0.0	0.0
H^4				14.0	5.8	7.7
H^5					8.0	5.2
H^6						13.4

[a] ^{1}H NMR spectrum acquired at 400 MHz in DMSO-d_6. [b] Nuclei H^2, H^4, and H^6 are interchangeable with nuclei H^3, H^5, and H^7.

Synthesis of the required linear precursor **4** was achieved using Fmoc-strategy solid phase peptide synthesis (Scheme 2).[12] Wang resin preloaded with Fmoc-L-phenylalanine (**5**) was subjected to an iterative deprotection/peptide coupling sequence, employing HBTU/Hünig's base as the coupling reagents, to give the fully assembled resin-bound heptapeptide **6**. The linear peptide was then cleaved from the resin, with simultaneous removal of the D-tryptophan side chain protecting group, to give **4** in relatively high purity and 96% overall yield. A long-range correlation between γ-H of valine #1 and α-H of valine #2 was observed in the ROESY spectrum of **4** (Scheme 2), suggesting that this peptide exhibits sufficient conformational flexibility to bring the head and tail proximal in space, at least transiently.[13] A single set of resonances were observed in the ^{1}H and ^{13}C NMR spectra of **4**, indicating that any conformational mobility is fast on the NMR time scale at room temperature.

Cyclization of **4** was performed at 5 mM in DMF, using DMTMM as the coupling reagent (Scheme 2).[14] Gratifyingly the cyclization reaction was found to be rapid and efficient, with LCMS analysis of the reaction mixture revealing that the starting material (m/z [MH$^+$] = 777) was completely consumed and replaced by a single product of mass consistent with the desired cyclic product (m/z [MH$^+$] = 759) within a reaction time of ten minutes. The target cyclic peptide **1** was isolated in 81% yield after preparative reverse-phase HPLC purification. No products arising from epimerization or cyclooligomerization side reactions were observed. The characterization data for synthetic **1**

dx.doi.org/10.1021/jo200813a |*J. Org. Chem.* 2011, 76, 5502–5505

(^{1}H NMR, ^{13}C NMR, HRMS, UV, CD) were in good agreement with those reported for the natural sample of **1**, confirming the original structural assignment of this metabolite.[5]

The ^{1}H NMR spectrum of **1** was analyzed in detail to probe the conformational characteristics of the GABA component. This segment of the molecule constitutes a seven-spin system, with each proton giving rise to a unique signal in the ^{1}H NMR spectrum (Table 1). The complex multiplets corresponding to each proton in the spin system were deconvoluted using a software-based simulation/iteration sequence.[15] It emerges that the $^3J_{HH}$ spin−spin coupling constants of the GABA residue are mostly intermediate in magnitude (Table 1),[16] confirming that this region of the natural product does indeed exhibit conformational flexibility.

In summary, the first total synthesis of unguisin A (**1**) is reported, confirming the structure of this natural product. A noteworthy feature of this synthesis is the rapid and efficient cyclization reaction of a GABA-containing linear precursor; this result may have broader implications in terms of a strategy for facilitating the synthesis of analogues of "difficult" cyclic peptides.[17] In the immediate context of unguisin A (**1**), this work will enable more thorough biological profiling to be performed, and it provides a platform for the production of non-natural analogues for structure−activity relationship studies, especially analogues that are conformationally constrained in the GABA region.[18]

EXPERIMENTAL SECTION

General Experimental Methods. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100 MHz, respectively, and detailed peak assignments were made with the aid of COSY, ROESY, DEPT, and HMBC experiments. All reagents and solvents were used as obtained commercially.

D-Valine−D-Alanine−D-Tryptophan−GABA−D-Alanine−D-Valine−L-Phenylalanine (4). Solid-phase peptide synthesis was conducted manually on 0.05 mmol scale in a sinter-fitted polypropylene syringe according to the following general procedures. *Resin preparation:* Wang resin (100−200 mesh) preloaded with Fmoc-L-phenylalanine (0.65 mmol/g) was immersed in DCM and agitated for 1 h, then drained and washed with DMF (5 × 1 min). *Fmoc deprotection:* The resin was agitated in 10% piperidine/DMF (3 × 3 min), then washed with DMF (3 × 1 min), DCM (3 × 1 min), and DMF (5 × 1 min). The collected deprotection solutions were diluted 100-fold with 10% piperidine/DMF, and the resin loading was estimated by measuring the absorbance of the piperidine−fulvene adduct at 301 nm (ε = 7800 M^{-1}cm^{-1}). *Peptide coupling:* A solution was prepared of the appropriate Fmoc-protected amino acid (3 equiv relative to resin loading) and HBTU (2.9 equiv) in minimal DMF. DIPEA (6 equiv) was added, and the resulting solution was immediately added to the resin and agitated for 1 h. The resin was drained and washed with DMF (3 × 1 min), DCM (3 × 1 min), and DMF (5 × 1 min). *Cleavage:* After the last Fmoc deprotection, the resin was washed with DCM (3 × 1 min) and then dried in vacuo. A solution of TFA/H_2O/triisopropylsilane (95:2.5:2.5) was added to the resin and agitated for 2 h. The resin was drained and washed with TFA (2 × 1 min). The combined cleavage solutions were concentrated in vacuo to give a clear glassy solid. Diethyl ether was added, and the supernatant decanted (4 ×). The residue was dried in vacuo to afford **4** (TFA salt) as a white solid (41 mg, 96% overall yield); mp 105−115 °C; $[\alpha]_D$ +24 (*c* 0.40, MeOH); IR (neat) ν_{max} (cm^{-1}) 3276, 1660, 1539; ^{1}H NMR (400 MHz, DMSO-d_6, COSY, ROESY) δ 10.77 (d, *J* = 2.0 Hz, 1H, Trp ArNH), 8.50 (d, *J* = 7.3 Hz, 1H, Ala #1 NH), 8.25 (d, *J* = 8.3 Hz, 1H, Phe NH), 8.07 (br s, 3H, Val #1 NH_3), 8.04 (d, *J* = 8.1 Hz, 1H, Trp NH), 7.95 (d, *J* = 7.5 Hz, 1H, Ala #2 NH), 7.90 (t, *J* = 5.5 Hz, 1H, GABA NH), 7.58 (d, *J* = 9.3 Hz, 1H, Val #2 NH), 7.55 (d, *J* = 8.0 Hz, 1H, Trp ArH4), 7.31 (d, *J* = 8.1 Hz, 1H, Trp ArH7), 7.26−7.15 (m, 5H, Phe ArH), 7.09 (d, *J* = 2.0 Hz, ArH2), 7.04 (dd, *J* = 8.1, 7.3 Hz, 1H, Trp ArH6), 6.95 (dd, *J* = 8.0, 7.3 Hz, 1H, Trp ArH5), 4.48−4.42 (m, 2H, Phe αH + Trp αH), 4.40 (dq, *J* = 7.3, 7.1 Hz, 1H, Ala #1 αH), 4.32 (dq, *J* = 7.5, 7.1 Hz, 1H, Ala #2 αH), 4.17 (dd, *J* = 9.3, 5.9 Hz, 1H, Val #2 αH), 3.59 (m, 1H, Val #1 αH), 3.09 (dd, *J* = 13.6, 4.4 Hz, 1H, Phe βH), 3.08−2.91 (m, 4H, Trp βH + Trp βH′ + 2 × GABA γH), 2.83 (dd, *J* = 13.6, 10.4 Hz, 1H, Phe βH′), 2.06−1.97 (m, 3H, Val #1 βH + 2 × GABA αH), 1.80 (m, 1H, Val #2 βH), 1.53 (m, 2H, GABA 2 × βH), 1.22 (d, *J* = 7.1 Hz, 3H, Ala #1 βH), 1.15 (d, *J* = 7.1 Hz, 3H, Ala #2 βH), 0.89 (d, *J* = 7.0 Hz, 3H, Val #1 γH), 0.88 (d, *J* = 6.8 Hz, 3H, Val #1 γH′), 0.64 (d, *J* = 6.8 Hz, 3H, Val #2 γH), 0.56 (d, *J* = 6.8 Hz, 3H, Val #2 γH′); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6, DEPT, HMBC) δ 172.9 (Phe C═O), 172.2 (Ala #2 C═O), 171.7 (GABA C═O), 171.4 (Ala #1 C═O), 170.9 (Trp C═O), 170.7 (Val #2 C═O), 167.4 (Val #1 C═O), 137.6 (Phe ArC1), 136.1 (Trp ArC7a), 129.1 (Phe ArC2/C3), 128.1 (Phe ArC4/C5), 127.4 (Trp ArC3a), 126.4 (Phe ArC6), 123.5 (Trp ArC2), 120.8 (Trp ArC6), 118.4 (Trp ArC4), 118.2 (Trp ArC5), 111.3 (Trp ArC7), 109.8 (Trp ArC3), 57.3 (Val #1 αC), 57.1 (Val #2 αC), 53.7 (Trp αC), 53.4 (Phe αC), 48.3 (Ala #1 αC), 48.1 (Ala #2 αC), 38.2 (GABA αC), 36.8 (Phe βC), 32.5 (GABA γC), 30.9 (Val #2 βC), 29.8 (Val #1 βC), 28.0 (Trp βC), 25.2 (GABA βC), 19.1 (Val #2 γC), 18.3 (Val #1 γC), 18.2 (Ala #1 βC), 17.8 (Ala #2 βC), 17.6 (Val #1 γC′), 17.3 (Val #2 γC′); HRMS (ESI, +ve) $C_{40}H_{57}N_8O_8^+$ [MH^+] requires *m*/*z* 777.4294, found 777.4301.

Unguisin A (1). To a solution of **4**·TFA (14.7 mg, 16.5 μmol) in DMF (2 mL) was added a solution of DMTMM·BF_4 (16.2 mg, 49.5 μmol) in DMF (0.94 mL) followed by DIPEA (10.3 μL, 59.4 μmol), and the resulting mixture was stirred at room temperature under a nitrogen atmosphere. At time intervals, an aliquot (2 μL) was withdrawn from the reaction mixture, diluted with 0.1% TFA/MeOH (80 μL), and directly analyzed by LCMS. After 5 h the reaction mixture was concentrated in vacuo, and the residue was subjected to preparative reverse-phase HPLC employing 0.1% TFA/H_2O as eluent A and 0.1% TFA/MeCN as eluent B (gradient: 100% A for 10 min, then ramped to 80% B over 40 min). The appropriate fraction (retention time = 32.6 min) was freeze-dried to give **1** as a fluffy white solid (10.1 mg, 81%); mp >250 °C; $[\alpha]_D$ +29.6 (289 nm, *c* 0.36, EtOH); IR (neat) ν_{max} (cm^{-1}) 3293, 1667, 1659, 1635, 1542, 1523, 1506, 1201, 1183, 1143; UV (EtOH) λ_{max} (log ε) 290 (3.60), 282 (3.65), 274 (3.63), 217 (4.40); CD (EtOH) λ ext ($\Delta\varepsilon$) 222 (4.72), 204 (−12.33); ^{1}H NMR (400 MHz, DMSO-d_6, COSY, ROESY) δ 10.82 (d, *J* = 1.8 Hz, 1H, Trp ArNH), 8.55 (d, *J* = 8.3 Hz, 1H, Phe NH), 8.40 (d, *J* = 4.6 Hz, 1H, Ala #1 NH), 8.11 (d, *J* = 4.2 Hz, 1H, Val #2 NH), 8.02 (d, *J* = 7.0 Hz, 1H, Trp NH), 7.87 (d, *J* = 9.7 Hz, 1H, Val #1 NH), 7.82 (d, *J* = 6.0 Hz, 1H, Ala #2 NH), 7.69 (dd, *J* = 6.2, 4.8 Hz, 1H, GABA NH), 7.53 (d, *J* = 7.8 Hz, 1H, Trp ArH4), 7.33 (d, *J* = 8.1 Hz, 1H, Trp ArH7), 7.24−7.17 (m, 4H, Phe ArH2,3,5,6), 7.13 (m, 1H, Phe ArH4), 7.11 (d, *J* = 1.8 Hz, 1H, Trp ArH2), 7.07 (dd, *J* = 8.1, 7.1 Hz, 1H, Trp ArH6), 6.98 (dd, *J* = 7.8, 7.1 Hz, 1H, Trp ArH5), 4.33 (ddd, *J* = 12.0, 8.3, 3.4 Hz, 1H, Phe αH), 4.22 (dq, *J* = 6.9, 6.0 Hz, 1H, Ala #2 αH), 4.09 (dd, *J* = 9.7, 9.7 Hz, 1H, Val #1 αH), 4.05 (dt, *J* = 7.6, 7.0 Hz, Trp αH), 3.95 (dq, *J* = 7.0, 4.6 Hz, 1H, Ala #1 αH), 3.49 (dd, *J* = 8.5, 4.2 Hz, 1H, Val #2 αH), 3.27 (dd, *J* = 13.1, 3.4 Hz, 1H, Phe βH), 3.19 (d, *J* = 7.1 Hz, 2H, 2 × Trp βH), 3.12 (dddd, *J* = 14.0, 7.0, 6.2, 6.0 Hz, 1H, GABA γH), 2.95 (dddd, *J* = 14.0, 7.0, 6.0, 4.8 Hz, 1H, GABA γH′), 2.60 (dd, *J* = 13.1, 12.0 Hz, 1H, Phe βH′), 2.10 (ddd, *J* = 13.4, 8.0, 5.8 Hz, 1H, GABA αH), 2.01 (ddd, *J* = 9.8, 6.8, 6.6 Hz, 1H, Val #1 βH), 1.96 (ddd, *J* = 13.4, 7.7, 5.2 Hz, 1H, GABA αH′), 1.69 (ddddd, *J* = 14.0, 7.7, 7.0, 7.0, 5.8 Hz, 1H, GABA βH), 1.61−1.51 (m, 2H, Val #2 βH + GABA βH′), 1.16 (d, *J* = 7.0 Hz, 3H, Ala #1 βH), 1.14 (d, *J* = 6.9 Hz, 3H, Ala #2 βH), 0.75 (d, *J* = 6.8 Hz, 3H, Val #1 γH), 0.73 (d, *J* = 6.7 Hz, 3H, Val #2 γH), 0.67 (d, *J* = 6.6 Hz, 3H, Val #1 γH′), 0.28 (d, *J* = 6.7 Hz, 3H, Val #2 γH′); ^{13}C {^{1}H} NMR (100 MHz, DMSO-d_6, DEPT, HMBC) δ 173.1 (Ala #2

dx.doi.org/10.1021/jo200813a |*J. Org. Chem.* 2011, 76, 5502–5505

C=O), 172.7 (Ala #1 C=O), 171.9 (GABA C=O), 171.63 (Val #1 C=O), 171.57 (Val #2 C=O), 171.2 (Trp C=O), 170.6 (Phe C=O), 138.5 (Phe ArC1), 136.3 (Trp ArC7a), 129.2 (Phe ArC2/3), 128.1 (Phe ArC4/5), 127.1 (Trp ArC3a), 126.2 (Phe ArC6), 123.8 (Trp ArC2), 121.1 (Trp ArC5), 118.4 (Trp ArC4 + Trp ArC6), 111.4 (Trp ArC7), 110.6 (Trp ArC3), 60.9 (Val #2 αC), 58.8 (Val #1 αC), 55.3 (Phe αC), 55.2 (Trp αC), 49.9 (Ala #1 αC), 47.9 (Ala #2 αC), 38.5 (GABA αC), 36.4 (Phe βC), 32.9 (GABA γC), 30.3 (Val #1 βC), 28.5 (Val #2 βC), 26.0 (GABA βC), 25.2 (Trp βC), 19.7 (Val #2 γC), 18.9 (Val #1 γC), 18.7 (Val #1 γC′), 18.4 (Val #2 γC′), 18.1 (Ala #2 βC), 17.3 (Ala #1 βC); MS (ESI, +ve) *m*/*z* 759 (MH^+, 100%), 781 (MNa^+, 10%); HRMS (ESI, +ve) $C_{40}H_{54}N_8O_7Na^+$ [MNa^+] requires *m*/*z* 781.4008, found 781.4004.

■ ASSOCIATED CONTENT

Supporting Information. Characterization data (NMR spectra, HPLC traces) of compounds **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was funded by a University of Sydney Postdoctoral Research Fellowship awarded to L.H.

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