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# Total Synthesis of Azumamide E and Sugar Amino Acid-Containing Analogue

Srivari Chandrasekhar,*[,†] Chennamaneni Lohitha Rao,[†] Mallikanti Seenaiah,[†] Police Naresh,[‡] Bharatam Jagadeesh,[‡] Dharani Manjeera,[§] Arpita Sarkar,[§] and Manika Pal Bhadra[§]

*Organic Division-I, Centre for Nuclear Magnetic Resonance, and Centre for Chemical Biology, Indian Institute of Chemical Technology, Hyderabad, India 500 607*

*srivaric@iict.res.in*

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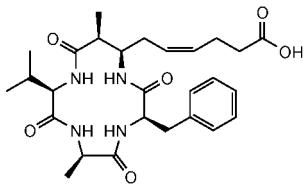
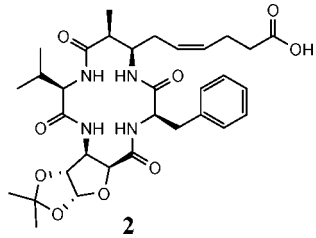


**1**

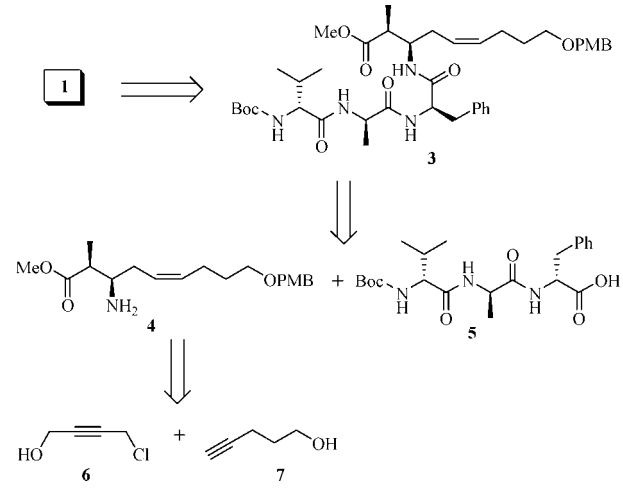


**2**

An efficient and practical total synthesis of marine cyclic tetrapeptide, natural product azumamide E (**1**) is achieved via high-yielding reactions. The strategy also allowed us to synthesize the azumamide E-SAA (sugar amino acid) analogue (**2**), whose solution-phase NMR and biological activity studies were also carried out.

The major concern in cancer chemotherapy has been the drug resistance. There have been several new chemical entities brought into clinic with various structural diversities and complexities to overcome this inherent drawback. More recently, the marine invertebrates have been providing some of the most interesting and complex secondary metabolites with diverse affinities toward biological targets. Among these, the most fascinating have been the cyclic peptides[1] with impressive antiproliferative properties. Fusetani et al. have isolated azumamides A−E from the marine sponge *Mycale izuensis*.[2] These natural products belong to a rare class of HDAC (histone deacetylase) inhibitors[3] incorporating four nonribosomal amino acid residues; three of them are α-amino acids (D-series), while the fourth one is a unique *β*-amino acid [(*Z*)-(2*S*,3*R*)-3-amino-2-methyl-5-nonenedioic acid-9-amide (Amnaa)] in azumamide A, B, and D and a free acid in C and E.

**SCHEME 1. Retrosynthetic Plan for Azumamide E**



The azumamide E, in particular, has shown histone deacetylase (HDAC) inhibition at the lowest concentration among all other congeners of azumamides.[4,5] We have been fascinated both by the structural elegance as it incorporates a *β*-amino acid skeleton and by the novel biological profile. Recently, we have synthesized novel *β*-amino acids as a part of our studies devoted to the understanding of the folding patterns of short peptide oligomers.[6] We embarked on the total synthesis of azumamide E (Scheme 1), while also being keen to incorporate one of our own *β*-amino acids (*β*-SAA)[6a] into the structural motif of azumamide, by replacing the D-alanine part. This novel analogue, azumamide E-SAA hybrid, is expected to show a better

† Organic Divison-1.
‡ Centre for Nuclear Magnetic Resonance.
§ Centre for Chemical Biology.

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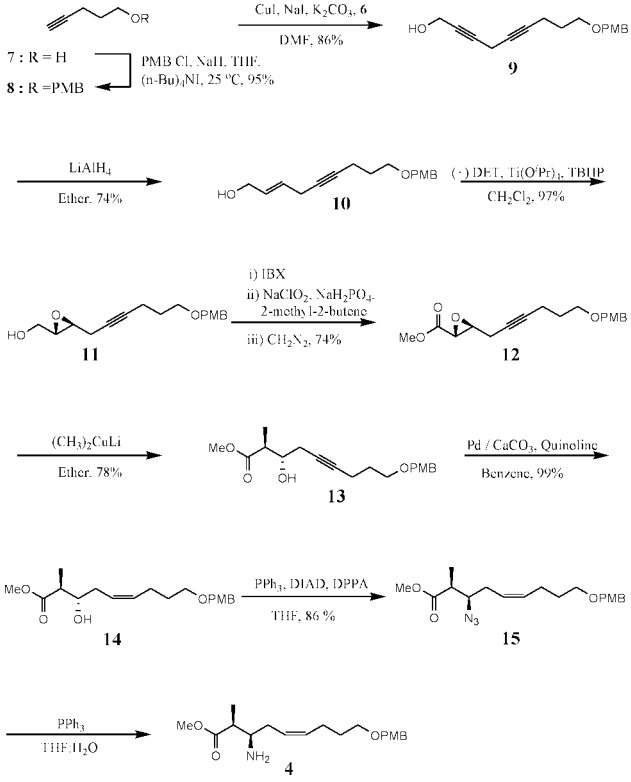
**SCHEME 2. Synthesis of β-Amino Acid Methyl Ester 4**

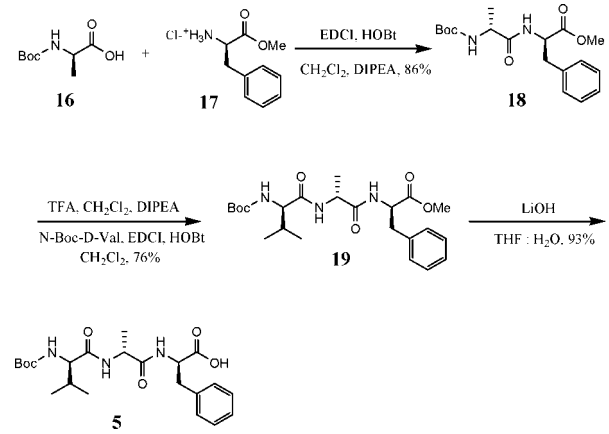



profile on the basis of the extensive reports of Kessler et al.[7] Herein, we report the total synthesis of azumamide E (**1**) and azumamide E-SAA (sugar amino acid) analogue (**2**) in a stereoselective manner.

The most logical disconnection of this scarce natural product made us visualize **4** and **5** as key intermediates. The synthesis of azumamide E-SAA **2** required **28** as a key intermediate, and the β-amino ester **4** is a common precursor for the total synthesis of both **1** and **2**.

The unusual β-amino acid methyl ester **4** was synthesized as shown in Scheme 2. The commercially available 4-pentyne-1-ol **7** was benzylated with PMBCl to realize the PMB ether **8** in over 95% yield. The chlorobutyne-1-ol **6** was coupled with **8** in the presence of CuI, NaI, and $K_2CO_3$ to generate the skipped bis(acetylene) **9** in 86% yield. The selective reduction of the propargylic alcohol functionality to (*E*)-allyl alcohol was achieved in 74% yield by $LiAlH_4$[8] as reducing agent. The Katsuki−Sharpless asymmetric epoxidation[9] with L-(+)-diethyl tartrate, $Ti(O\text{-}i\text{-}Pr)_4$, and TBHP was uneventful with 97% yield and over >98% ee. The three-step oxidation−esterfication was done in one stroke, without isolating the intermediates, to furnish the epoxy ester **12** in 74% overall yield for three steps. The higher-order methyl cuprate was generated following literature procedure[10] and was added to the epoxy ester to realize the single regioisomer **13** in 78% yield. The hydrogenation of **13** using Lindlar's catalyst[11] gave the (*Z*)-homoallyl alcohol **14** in quantitative yields. This, on further reaction with DPPA under Mitsunobu conditions,[12] generated the azido derivative **15** in 86% yield with inversion of configuration. The azido group in **15** was reduced under Staudinger reaction conditions[13] ($PPh_3$, $THF/H_2O$) to furnish the β-amino acid methyl ester **4**. This is a key intermediate in the total synthesis of both targets. The synthetic route described so far is well-standardized, and multigram materials are obtained rather routinely.

**SCHEME 3. Synthesis of Trimer Acid 5**




The two other building blocks were synthesized following the standard peptide synthesis protocol. The *N*-Boc-D-alanine **16** was coupled with the methyl ester of phenyl alanine **17** using EDCI/HOBt to get the dipeptide **18** (86% yield). This was further coupled with *N*-Boc-D-valine, after removal of the Boc group in **18**, to generate the tripeptide **19** that, on LiOH hydrolysis, gave acid **5** (Scheme 3). This last compound was amidated with amine **4** in the presence of EDCI/HOBt and $CH_2Cl_2$ to furnish **3**. A further reaction with TBSOTf and 2,6-lutidine as base furnished silyl carbamate **20**. We then performed a LiOH-mediated double hydrolysis of silyl carbamate and methyl ester functionalities to provide free amino acid **21** that was ready for the macrolactamization. Luckily, the first attempted EDCI/HOBt method was successful to realize the protected azumamide **22** in 79% yield. The cleavage of MPM ether under oxidative conditions (DDQ, $CH_2Cl_2/H_2O$) provided the free alcohol **23** that, after oxidation with BAIB and TEMPO,[14] afforded efficiently the target compound **1** in 84% yield (Scheme 4). Thus, we were able to demonstrate the total synthesis of a very potent natural product azumamide E via a high-yielding strategy (overall ∼11% yield).

Inspired by the publication of Kessler et al.,[7] wherein the somatostain analogues incorporating β-amino acid (derived from furanose sugar) have induced apoptosis in multi-drug-resistant cells, we embarked on the synthesis of a similar analogue, as the sugar β-amino acid methyl ester **24** was readily available in our repertoire of building blocks. The building block **24** was amidated with *N*-Boc-D-valine under well-established coupling conditions to produce **25** in 85% yield, which, on hydrolysis with LiOH, gave acid **26** (Scheme 5). The tripeptide acid **28** was assembled by incorporating the methyl ester of D-phenyl alanine into **26** via **27** in two steps. The unusual β-amino acid methyl ester **4** was reacted with **28**, and the series of reactions

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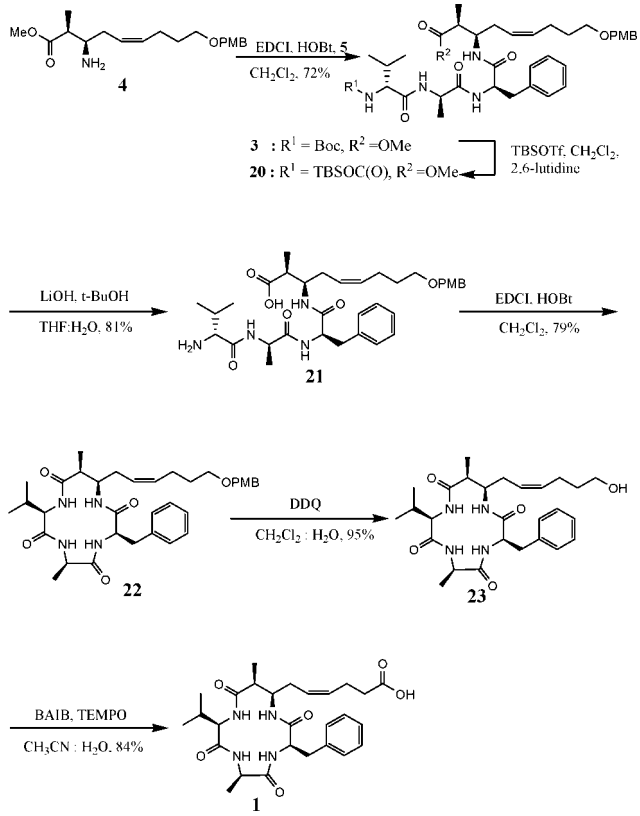
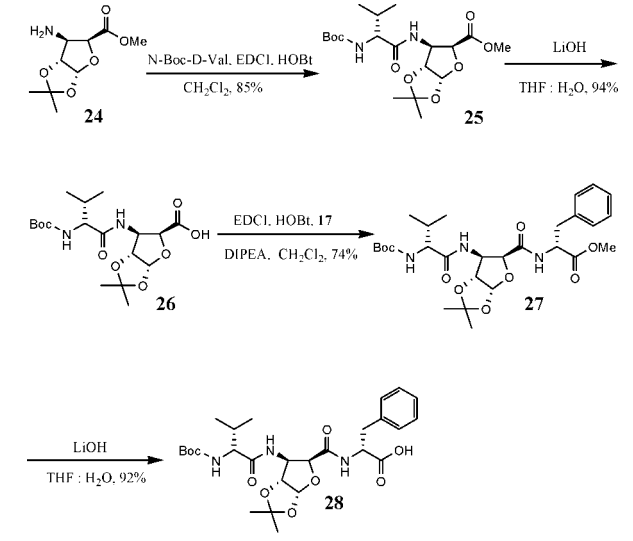
**SCHEME 4. Coupling of Amine 4 and Acid 5 and Completion of the Synthesis of Azumamide E**




**SCHEME 5. Synthesis of Trimer Acid 28**



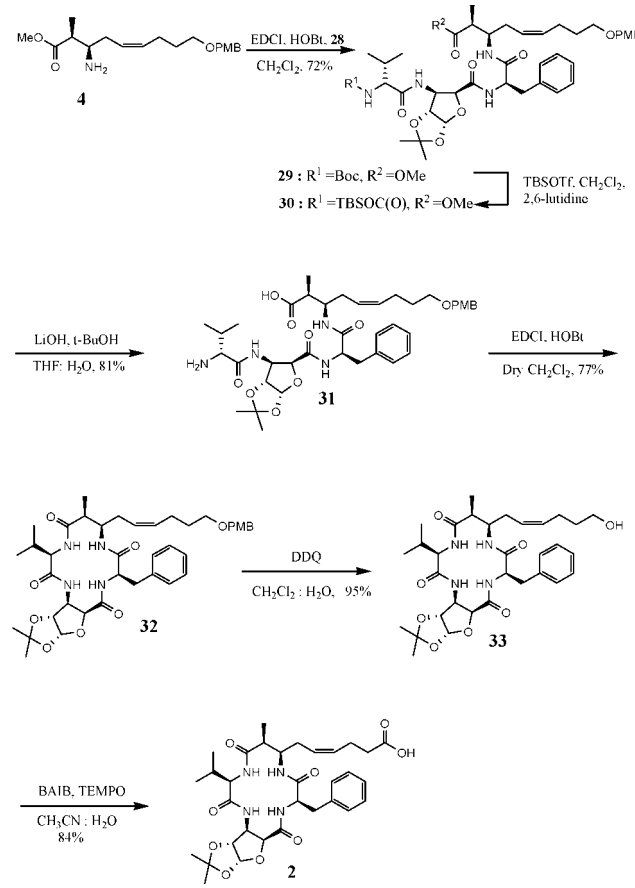

were performed similar to the ones described in Scheme 4 to synthesize for the first time azumamide E-SAA **2** in ~10.3% overall yield (Scheme 6).

To get an insight into the solution-state conformation for azumamide E-SAA **2** (Figure 1), the restrained MD calculations were carried out following simulated annealing protocol.

Superposition of energy-minimized structures derived from these MD studies showed good convergence,[15] where at least the backbone has been well-defined, suggesting a predominantly

(15) See Supporting Information.

**SCHEME 6. Coupling of Acid 28 and Amine 4 and Completion of the Synthesis of Azumamide E-SAA Analogue 2**




single conformation for **2** during the time scale of observation. Assignment of side chain protons was difficult because of resonance overlaps in the $^1$H spectrum, which have limited us to use only 10 spatial restraints in MD. Despite the flexibility in the side chain, the orientation is restricted over the peptide cavity, which was evidenced in simulated annealing structures and supported by the observed NOEs between $\beta$ SAA−$C_\delta$H and methylene protons of Amnda. The temperature coefficient studies $\Delta\delta/\Delta T > 4$ for all NHs excluded the possibility of hydrogen bonding and, thus, any defined secondary structure. This solution structure of the sugar analogue is different from its natural counterpart in orientation of the side chain.[5a]

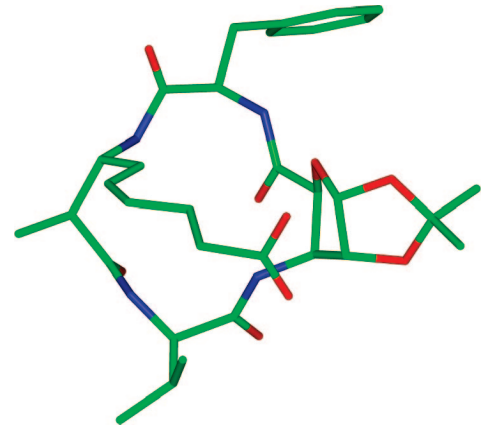


**FIGURE 1.** NMR solution-state ($CD_3OH$) conformation of azumamide E-SAA **2** obtained by restrained molecular dynamics calculations.

**TABLE 1. Biological Activities of Compounds TSA, 1, and 2 on HeLa NE**

| compounds | deacetylated lysine/ abs HDAC($\mu$M) | % of HDAC inhibition[a] |
|---|---|---|
| **TSA** (10 $\mu$M) | 62.77 ± 2.71 | 37 |
| **TSA** (20 $\mu$M) | 25.53 ± 1.08 | 74 |
| **1** (10 $\mu$M) | 81.24 ± 2.19 | 19 |
| **1** (20 $\mu$M) | 29.61 ± 1.23 | 71 |
| **2** (10 $\mu$M) | 52.01 ± 1.59 | 48 |
| **2** (20 $\mu$M) | 4.3 ± 0.019 | 96 |

[a] HeLa nuclear extract without any HDAC inhibitor, which shows 100 $\mu$M of deacetylated lysine and 0% HDAC inhibition.

To characterize whether the compounds are reliable and potential histone deacetylase inhibitors, the effect of the compounds on HDAC activity was determined by in vitro HDAC enzyme assays. Trichostatin A (TSA), a known histone deacetylase inhibitor, was used as positive control to compare the activity of compounds **1** and **2**. Parallel to its effect on cell cycle arrest, compounds **1** and **2** were found to inhibit cellular histone deacetylase activity derived from cell lysate directly. The histone deacetylase activity was almost completely inhibited by the 20 $\mu$M compound **2** (Table 1) in a cell-free system.

In summary, we have demonstrated a successful synthesis of azumamide E **1** and azumamide E-SAA **2** involving a solution-phase peptide synthesis strategy in good overall yields. The preliminary biological data of azumamide E-SAA **2** was found to be superior over the synthetic natural product **1**.

## Experimental Section

**(*Z*)-6-((2*R*,5*R*,8*R*,11*R*,12*S*)-8-Benzyl-2-isopropyl-5,12-dimethyl-3,6,9,13-tetraoxo-1,4,7,10-tetraazacyclotridecan-11-yl)hex-4-enoic Acid (Azumamide E) (1).** To a solution of **23** (30 mg, 0.06 mmol, 1 equiv) in a 1:1 mixture of $CH_3CN/H_2O$ (0.4 mL) were added TEMPO (3 mg, 0.02 mmol, 0.3 equiv) and BAIB (57 mg, 0.18 mmol, 3 equiv). The reaction mixture was stirred at ambient temperature for 6 h and then quenched by the addition of an aqueous $Na_2S_2O_3$ solution (1.5 mL). The aqueous phase was then extracted with $CHCl_3$ (5 × 5 mL), and the combined organic extracts were dried ($Na_2SO_4$) and concentrated in vacuo. Silica gel column chromatography (MeOH−$CH_2Cl_2$, 10:90) of the residue gave **1** (26 mg, 84%) as a white solid: mp 257−259 °C; $[\alpha]_D = +72.0$ (*c* 0.1, MeOH); [Lit. isolation[2] $[\alpha]_D = +53.0$ (*c* 0.06, MeOH); Lit. synthesis[5a] $[\alpha]_D = +58.2$ (*c* 0.06, MeOH); Lit. synthesis[5b] $[\alpha]_D = +84.7$ (*c* 0.525, MeOH)]; $^1$H NMR (600 MHz, $CD_3OH$) $\delta$ 9.50 (br s, 1H), 9.33 (br s, 1H), 8.20 (d, $J = 7.9$ Hz, 1H), 7.83 (br s, 1H), 7.31−7.16 (m, 5H), 5.61−5.54 (m, 1H), 5.36−5.29 (m, 1H), 4.52−4.45 (m, 1H), 4.35 (br t, $J = 7.4$ Hz, 1H), 4.32−4.27 (m, 1H), 3.94 (t, $J = 9.5$ Hz, 1H), 3.11−3.00 (m, 2H), 2.90−2.66 (m, 3H), 2.44−2.37 (m, 1H), 2.36−2.21 (m, 4H), 1.35 (d, $J = 6.3$ Hz, 3H), 1.33 (d, $J = 6.6$ Hz, 3H), 1.04 (d, $J = 6.6$ Hz, 3H), 1.00 (d, $J = 6.6$ Hz, 3H); $^{13}$C NMR (100 MHz, $CD_3OH$) $\delta$ 176.9 (2C), 175.0, 174.6, 174.0, 138.4, 133.1, 130.1 (2C), 129.2 (2C), 127.6, 126.8, 65.0, 58.5, 52.6, 52.5, 44.9, 38.0, 37.9, 30.0, 28.8, 25.3, 20.0, 19.3, 16.1, 11.8; HRMS (ESIMS) calcd for $C_{27}H_{38}N_4O_6Na$ 537.2689 $[M + Na]^+$, found 537.2678 $[M + Na]^+$.

**Azumamide E-SAA (2).** The oxidation procedure described above to obtain **1** from **23** was applied to **33** (15 mg, 0.024 mmol) with BAIB (23 mg, 0.073 mmol) and TEMPO (1.1 mg, 0.0073 mmol), followed by silica gel column chromatography (MeOH−$CH_2Cl_2$, 11:89), yielding **2** (12 mg, 82% yield) as a white solid: mp 142−146 °C; $[\alpha]_D = +93.0$ (c 0.17, MeOH); $^1$H NMR(500 MHz, $CD_3OH$) $\delta$ 8.45 (d, $J = 7.7$ Hz, 1H), 8.31 (br s, 1H), 7.93 (d, $J = 6.9$ Hz, 1H), 7.48 (br s, 1H), 7.27−7.14 (m, 5H), 6.06 (d, $J = 3.8$ Hz, 1H), 5.49−5.42 (m, 1H), 5.36−5.29 (m, 1H), 4.57−4.51 (m, 2H), 4.47 (d, $J = 3.1$ Hz, 1H), 4.11−4.02 (m, 1H), 3.48 (t, $J = 8.5$ Hz, 1H), 3.31−3.30 (m, 1H), 3.08 (dd, $J = 13.9$, 4.6 Hz, 1H), 2.93 (dd, $J = 13.9$, 10.1 Hz, 1H), 2.81−2.73 (m, 1H), 2.72−2.64 (m, 1H), 2.47−2.28 (m, 6H), 1.41 (s, 3H), 1.28 (s, 3H), 1.26 (d, $J = 7.3$ Hz, 3H), 0.90 (d, $J = 6.9$ Hz, 6H); $^{13}$C NMR (100 MHz, $CD_3OH$) $\delta$ 179.9, 179.8, 177.0, 175.9, 172.2, 140.8, 134.1, 132.6 (2C), 132.0 (2C), 130.6, 130.3, 116.2, 109.2, 88.4, 82.9, 67.7, 60.2, 55.3, 46.3, 41.3, 37.5, 33.2, 32.6, 30.9, 29.4, 28.8, 26.5, 22.5 (2C), 15.6; ESI (MS) 651 $[M + Na]^+$; HRMS (ESI) calcd for $C_{32}H_{44}N_4O_9Na$ 651.3000 $[M + Na]^+$, found 651.3005 $[M + Na]^+$.

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**Supporting Information Available:** General experimental procedures and spectroscopic data for the compounds and copies of $^1$H NMR and $^{13}$C NMR spectra are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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