EurJOC European Journal of Organic Chemistry

FULL PAPER

DOI: 10.1002/ejoc.201403237

Synthesis of Tetrasubstituted Symmetrical Pyrazines from β-Keto γ-Amino Esters: A Mild Strategy for Self-Dimerization of Peptides

Mothukuri Ganesh Kumar,[a] Varsha J. Thombare,[a] Rupal D. Bhaisare,[a] Anindita Adak,[a] and Hosahudya N. Gopi*[a]

Keywords: Amino acids / Peptides / Oxidation / Cyclization / Nitrogen heterocycles

A facile synthesis of highly symmetrical tetrasubstituted pyrazines through simple aerial oxidation of β-keto γ-amino esters is reported. The scope of the reaction was examined by use of various amino acid side-chain functional groups and peptides. The mild and efficient transformation of β-keto γ-amino esters into pyrazines may serve as an attractive strategy for self-dimerization of peptides.

Introduction

Pyrazines are a class of heterocyclic aromatic compounds found ubiquitously in nature. Many of these natural pyrazine derivatives and their synthetic counterparts have shown excellent biological activities, including anticancer,[1] antiviral,[2] and antituberculosis[3] properties etc. Furthermore, the roles of tetrasubstituted pyrazines as sex pheromones in female insects[4] and as alarm pheromones in ponerine ants[5] have been documented. Some sexually deceptive orchid species produce pyrazines as semiochemicals to attract male wasps as pollinators.[4] Many bacterial strains use various substituted pyrazines as a sole source of carbon and energy.[6] Besides their biological activities, pyrazines have also been extensively used in the food and perfume industries, due to their taste and aromas.[7] In addition, pyrazine heterocycles have also been finding applications in materials science.[8] Some naturally occurring pyrazine derivatives – tetramethylpyrazine,[9] a representative barrenazine,[10] and cephalostatin[1a] – are shown in Figure 1. The majority of the natural pyrazines are derived from amino acids.[11] Recently, Kutonovas et al. delineated the possible catabolic pathway for the degradation of pyrazines.[12]

As a consequence of the growing demand for substituted pyrazine derivatives in the pharma, food, and perfume industries, various synthetic protocols have been developed. Generally, pyrazines are synthesized variously by condensation of 1,2-diamines with 1,2-dicarbonyl compounds,[13] self-condensation of α-amino ketones,[11d,14] or through reactions between α-haloketones and ammonia.[15] Besides

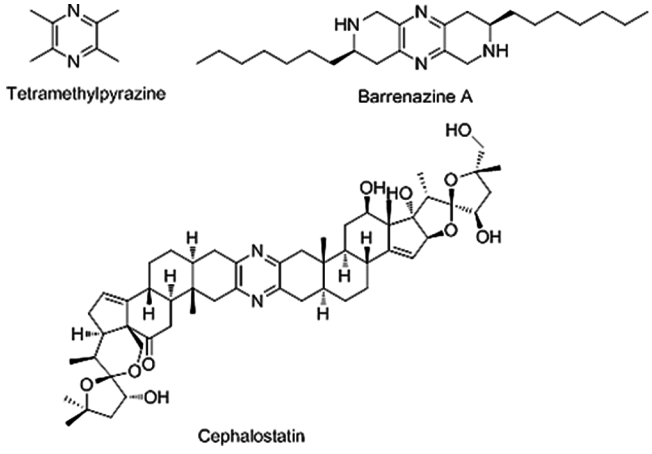


Figure 1. Examples of naturally occurring pyrazine derivatives.[9,10,1a]

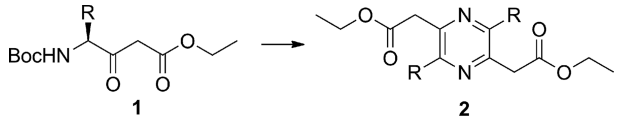
these, various other protocols involving α-haloenol acetates,[16] nitroepoxides,[17] ruthenium-catalyzed dehydrogenative coupling thermal treatment of azirine phosphonates and β-amino alcohols,[18] tosylketoximes,[19] and condensation of amino diketones with hydroxylamines[20] etc. have been developed. Recently, Sperry and colleagues examined the self-condensation of various amino aldehydes to produce substituted pyrazines; the study revealed the importance of Cbz protection over Boc and Fmoc protection for the amino aldehydes.[21] In addition, Rojas et al. reported the self-condensation of α-amino aldehydes to pyrazines in a three-step one-pot procedure.[22] In an accidental discovery, Pettit et al. also observed the formation of tetrasubstituted pyrazine from the methyl ester of β-keto γ-leucine as a byproduct in the total synthesis of respirantin.[23]

Recently, we reported the facile synthesis of β-keto γ-amino esters and their utility in the synthesis of β-hydroxy-γ-amino acids (statines) as well as fluorescent coumarin

[a] Department of Chemistry, Indian Institute of Science Education and Research,
Dr. Homi Bhabha Road, Pune-411 008, India
E-mail: hn.gopi@iiserpune.ac.in
www.iiserpune.ac.in

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201403237.

amino acids.[24] The wide applications of pyrazine derivatives motivated us to investigate the synthesis of tetrasubstituted pyrazines from β-keto γ-amino esters (Scheme 1). Here we report the mild synthesis of highly symmetrical tetrasubstituted pyrazines from β-keto γ-amino esters through a simple aerial oxidation. Through the utilization of this mild method for aromatization of β-keto esters, a new strategy for the self-dimerization of peptides has been developed.



Scheme 1. Tetrasubstituted pyrazines from β-keto γ-amino esters.

Results and Discussion

In order to establish whether β-keto γ-amino esters can undergo self-condensation and subsequent aerial oxidation to give pyrazines, we synthesized ethyl esters of various β-keto γ-amino acids, starting from *N*-Boc-protected amino aldehydes and ethyl diazoacetate in the presence of anhydrous tin chloride.[24a,25] A schematic representation of the reaction is shown in Scheme 2. All ethyl esters of β-keto γ-amino esters were isolated in good yields after column purification. To achieve the formation of pyrazine derivatives, we initially subjected β-keto γ-phenylalanine ethyl ester **1a** to the self-condensation reaction in the open air after the removal of the *N*-Boc group. The TFA salt of the γ-amino β-keto ethyl ester was dissolved in THF and neutralized with DIPEA (Scheme 3). The transformation of the γ-amino β-keto ester into tetrasubstituted pyrazine **2a** was achieved after the reaction mixture had been stirred overnight. Pure **2a** was isolated in good yield (75%) after column chromatography. Further, the pure **2a** gave X-ray-quality single crystals, and its structure is shown in Figure 2. We further examined whether Boc-phenylalanal can undergo similar self-condensation and subsequent aromatization after Boc group removal. Unlike the β-keto γ-amino ester, the α-amino aldehyde did not undergo cyclization and subsequent aromatization.[21]

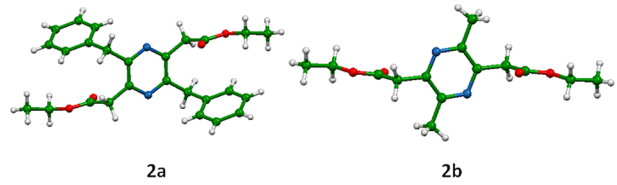
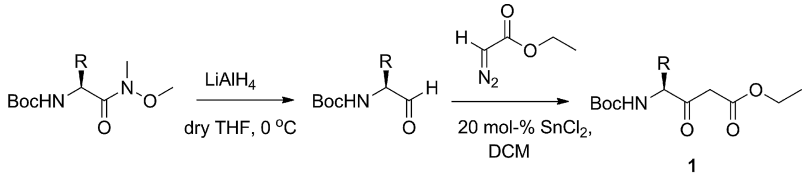


Figure 2. X-ray structures of **2a** and **2b**.

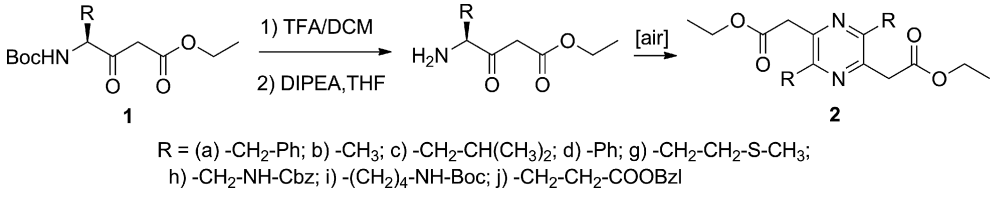
Inspired by the smooth aromatization of the unprotected β-keto ester of **1a** and the highly symmetric nature of pyrazine **2a**, we subjected other hydrophobic γ-amino β-keto esters **1b**–**1f** to the pyrazine synthesis. Although the β-keto esters **1e** and **1f**, derived from the β-branched amino acids Val and Ile, respectively, did not react, the others – **1b** to **1d** – gave the corresponding pyrazine products **2b**–**2d** in moderate to good yields. We did not isolate pyrazine derivatives from **1e** or **1f**. In addition to **2a**, **2b** also gave single crystals, and its X-ray structure is also shown in Figure 2.

The scope of the reaction was investigated further with γ-amino β-keto esters **1g**–**1j**, containing side-chain functional groups. We observed no interference of ester, thioether, or amide functional groups in the self-condensation and subsequent aromatization. All pyrazine products **2g**–**2j** were isolated in moderate to good yields and are listed in Table 1.

Inspired by the mild and biocompatible self-condensation and the aromatization of β-keto esters containing amine and carboxylic acid side-chains, we sought to investigate whether this strategy can be further extended to peptides. In addition, nature selectively utilizes α-amino ketones in the synthesis of symmetrical cephalostatins (Figure 1) and ritterazines.[1c,26] We anticipated that, as in the case of cephalostatins, it should also be possible to "self-staple"

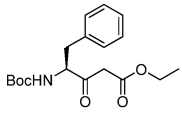
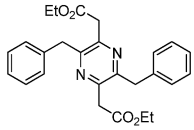
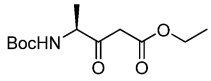
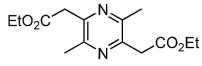
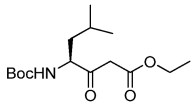
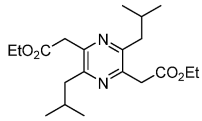
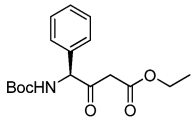
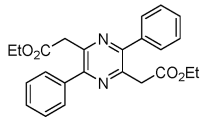
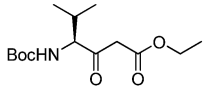
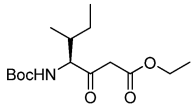
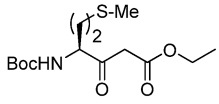
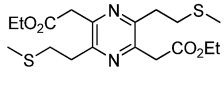
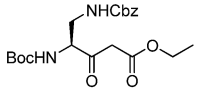
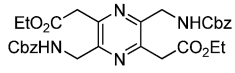
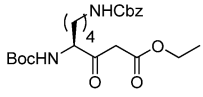
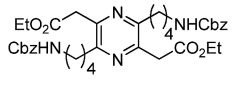
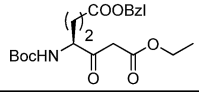
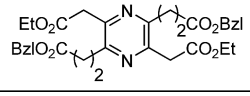


Scheme 2. Synthesis of *N*-Boc β-keto γ-amino esters starting from amino acids.



Scheme 3. Schematic representation of the synthesis of tetrasubstituted pyrazines starting from *N*-Boc β-keto γ-amino esters.

Table 1. List of pyrazines synthesized from β-keto γ-amino esters.

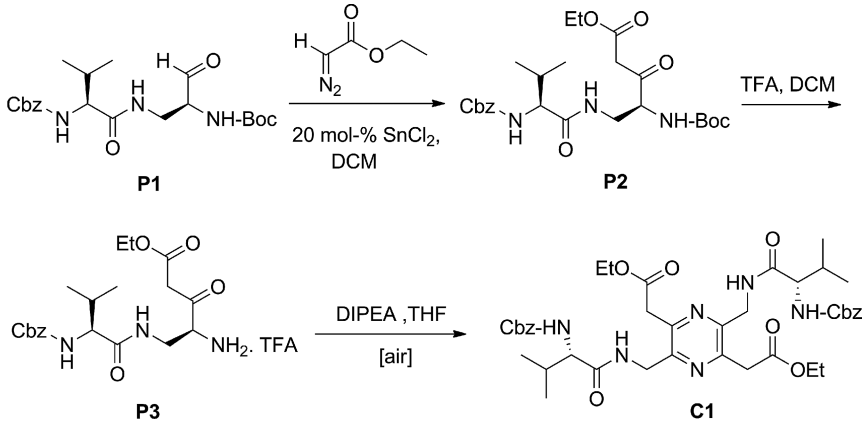
	β-Keto esters **1**	Pyrazines **2**	% Yield
a			75
b			67
c			60
d			62
e		not observed	
f		not observed	
g			67
h			63
i			78
j			60

peptides through aerial oxidation, if the side-chain amino groups of **1h** and **1i** and the carboxylic acid side-chain of **1j** were coupled with peptides.

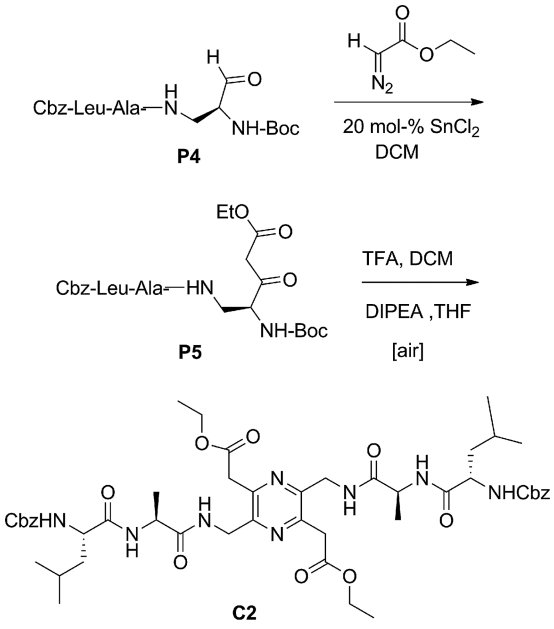
To verify this, we designed a new strategy for the synthesis of peptide β-keto esters, as shown in Scheme 4. The free amine in the Boc-Dap side-chain was directly coupled to the *N*-hydroxysuccinimide ester of *N*-Cbz-Val to give a dipeptide carboxylic acid. The free carboxylic acid was converted to afford the corresponding dipeptide aldehyde through LAH reduction of the corresponding Weinreb amide. The dipeptide aldehyde **P1** was transformed into β-keto ester **P2** by treatment with ethyl diazoacetate in the presence of tin(II) chloride. The pure dipeptide β-keto ester **P2** was isolated in good yield after the column purification. The Boc group of the dipeptide **P2** was selectively removed with TFA, and the TFA salt **P3** was neutralized with DIPEA as base. The free dipeptide β-keto ester was stirred overnight in open air. Conversion of dipeptide β-keto ester into pyrazine **C1** was observed within 12 h. The pure peptide pyrazine derivative **C1** was isolated after column chromatography in 55% yield.

We further extended this strategy to a tripeptide. A schematic representation of the synthesis of a tripeptide β-keto ester and the subsequent transformation of this β-keto ester into a pyrazine is shown in Scheme 5. The tripeptide aldehyde **P4** obtained after treatment of a Weinreb amide with LAH was converted into the corresponding β-keto ester **P5** by treatment with ethyl diazoacetate in the presence of anhydrous tin(II) chloride in CH_2Cl_2.

The tripeptide β-keto ester was subjected to the same aerial oxidation after removal of the Boc group as described earlier. The reaction was monitored by MALDI-TOF mass spectrometry, and the transformation of the β-keto ester into the corresponding pyrazine **C2** was observed within 12 h. We further noticed that an additional supply of oxygen from a balloon increased the rate of formation of pyrazine products from peptides, but it is not essential. The tripeptide pyrazine derivative **C2** was isolated in 50% yield after reversed-phase HPLC purification on a C_{18} column with a methanol/H_2O gradient system. These results clearly



Scheme 4. Synthesis of a dipeptide β-keto ester and subsequent pyrazine synthesis.



Scheme 5. Transformation of a tripeptide β-keto ester into a pyrazine.

suggest that, like the γ-amino-β-keto esters, peptide β-keto esters can also undergo self-condensation and subsequent aromatization to give pyrazine derivatives. The strategy described here provides a unique means for self-dimerization of peptides under very mild conditions.

Conclusions

Overall, we have demonstrated the conversion of ethyl esters of β-keto γ-amino acids into highly symmetrical tetrasubstituted pyrazines under very mild conditions. With the exceptions of the γ-amino β-keto esters derived from the sterically hindered amino acids valine and isoleucine, all other β-keto esters gave the corresponding tetrasubstituted pyrazines in moderate to good yields. In addition to the β-keto amino esters, we also demonstrated the synthesis of peptide β-keto esters and their subsequent transformation into highly symmetrical tetrasubstituted pyrazines. We are currently extending this new peptide "self-stapling" strategy to solid-phase synthesis. The mild and biocompatible aromatization of β-keto esters of amino acids and peptides reported here may find applications in medicinal chemistry and materials science.

Experimental Section

General Experimental Details: Solvent THF was dried with sodium and immediately distilled prior to use. Dichloromethane (CH_2Cl_2) was distilled prior to use. Column chromatography was performed with silica gel (120–200 mesh). ^1H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz respectively, with use of residual solvent as internal standards ($CDCl_3$). Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are reported in Hz. Mass spectra were recorded by MALDI TOF/TOF and Electron Spray Ionization (ESI).

General Procedure for the Synthesis of *N*-Protected γ-Amino β-Keto Esters: The appropriate *N*-protected amino aldehyde (2.0 mmol) was dissolved in CH_2Cl_2 (15 mL) at room temperature, and then tin(II) chloride (0.0756 g, 20 mol-%) was added, followed by ethyl diazoacetate (0.239 g, 2.1 mmol). Immediate gas evolution was observed. The reaction mixture was stirred and the progress of the reaction was monitored by TLC. After completion of the reaction (ca. 30 min), it was quenched with HCl (0.5 N, 10 mL) and the reaction mixture was extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was washed with brine (20 mL), dried with anhydrous sodium sulfate, and concentrated under reduced pressure to afford a greenish oily crude product, which was purified by silica gel column chromatography.

Ethyl (*S*)-4-[(*tert*-Butoxycarbonyl)amino]-3-oxo-5-phenylpentanoate (1a):[24a] White crystals (0.521 g, 78%). $[\alpha]_D^{25}$ = –54.5 (c = 0.6, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ = 12.16 (s, 1 H, enolic 17%), 7.24–7.15 (m, 5 H), 5.03–5.01 (d, J = 7.3 Hz, 1 H), 4.57–4.52 (q, J = 6.4 Hz, 1 H), 4.18–4.12 (q, J = 7.2 Hz, 2 H), 3.51–3.40 (dd, J = 16, 11.4 Hz, 2 H), 3.15–2.95 (m, 2 H), 1.38 (s, 9 H), 1.26–1.22 (t, J = 7.2 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.9, 166.8, 155.1, 136.0, 129.24, 128.6, 127.0, 80.2, 61.4, 60.4, 46.8,36.8, 28.2, 14.0 ppm. HRMS: calcd. for $C_{18}H_{25}NO_5$ [M + Na]$^+$ 358.1630; found 358.1633.

Ethyl (*S*)-4-[(*tert*-Butoxycarbonyl)amino]-3-oxopentanoate (1b):[24a] Light yellow liquid (0.398 g, 76%). $[\alpha]_D^{25}$ = –35.69 (c = 1, MeOH). ^1H NMR (500 MHz, $CDCl_3$): δ = 12.13 (s, 1 H), 5.17 (s, 1 H), 4.43–4.37 (m, 1 H), 4.24–4.20 (q, J = 7 Hz, 2 H), 3.62–3.54 (dd, J = 14.5, 10.5 Hz, 2 H), 1.46 (s, 9 H), 1.38–1.36 (d, J = 6.5 Hz, 3 H), 1.31–1.28 (t, J = 7 Hz, 3 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 202.5,166.9, 155.1, 80.1, 61.5, 55.4, 45.9, 28.3, 17.1, 14.1 ppm. HRMS: calcd. for $C_{12}H_{21}NO_5$ [M + Na]$^+$ 282.1317; found 282.1317.

Ethyl (*S*)-4-(*tert*-Butoxycarbonylamino)-6-methyl-3-oxoheptanoate (1c):[24a] Light yellowish liquid (0.476 g, 79%). $[\alpha]_D^{25}$ = –53.70 (c = 1, MeOH). ^1H NMR (500 MHz, $CDCl_3$): δ = 12.10 (s, 1 H), 4.96–4.94 (d, J = 9.5 Hz, 1 H), 4.40–4.36 (m,1 H), 4.24–4.20 (q, J = 7 Hz, 2 H), 3.63–3.53 (dd, J = 16.0, 18.5 Hz, 2 H), 1.74–1.67 (m, 3 H), 1.46 (s, 9 H), 1.32–1.29 (t, J = 7 Hz, 3 H), 0.97 (b, s, 6 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 203.02, 167.07, 155.55, 80.15, 61.51, 58.19, 46.35, 39.90, 28.31, 24.83, 23.28, 21.59, 14.12 ppm. HRMS: calcd. for $C_{15}H_{27}NO_5$ [M + Na]$^+$ 324.1786; found 324.1784.

Ethyl (*S*)-4-[(*tert*-Butoxycarbonyl)amino]-3-oxo-4-phenylbutanoate (1d): Yellowish liquid (0.46 g, 72%). $[\alpha]_D^{25}$ = –4.0 (c = 1, MeOH). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.29 (m, 5 H), 5.86 (d, J = 4 Hz, 1 H), 5.44 (d, J = 8 Hz), 4.09 (q, J = 8 Hz, 2 H), 3.39 (dd, J = 16, 60 Hz, 2 H), 1.39 (s, 9 H), 1.19 (t, J = 8 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 198.5, 166.3, 154.8, 135.9, 129.4, 128.9, 128.2, 80.2, 64.4, 61.6, 46.3, 28.3, 14.3 ppm. HRMS: calcd. for $C_{17}H_{23}NO_5$ [M + Na]$^+$ 344.1468; found 344.1469.

Ethyl (*S*)-4-(*tert*-Butoxycarbonylamino)-5-methyl-3-oxohexanoate (1e):[24a] Colorless liquid (0.48 g, 84%). $[\alpha]_D^{25}$= –32.64 (c = 1, MeOH). ^1H NMR (500 MHz, $CDCl_3$): δ = 12.11 (s, 1 H, enolic form, 6.5%), 5.06 (br. s, 1 H), 4.35–4.32 (m, 1 H), 4.22–4.18 (q, J = 7 Hz, 2 H), 3.57–3.50 (dd, J = 15.5, 3 Hz, 2 H), 2.27–2.23 (m, 1 H), 1.44 (s, 9 H), 1.29–1.26 (t, J = 7 Hz, 3 H), 1.02–0.82 (m, 6 H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 202.23, 166.75, 155.86, 80.03, 64.38, 61.55, 47.14, 29.56, 28.31, 19.84, 16.67, 14.10 ppm. HRMS: calcd. for $C_{14}H_{25}NO_5$ [M + Na]$^+$ 310.1630; found 310.1620.

Ethyl (4*S*,5*R*)-4-[(*tert*-Butoxycarbonyl)amino]-5-methyl-3-oxoheptanoate (1f):[24a] Colorless liquid (0.453 g, 76%). $[\alpha]_D^{25}$ = –25.08 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.09 (s, 1 H, enolic 7.5%), 5.03–5.01 (d, *J* = 8.24 Hz, 1 H), 4.31–4.28 (m, 1 H), 4.19–4.13 (q, *J* = 6.88 Hz, 2 H), 3.51 (s, 2 H), 1.97–1.90 (m, 1 H), 1.63–1.57 (m, 2 H), 1.41 (s, 9 H), 1.27–1.23 (t, *J* = 7.2 Hz, 3 H), 0.97–0.95 (dd, *J* = 3.64, 3.24 Hz, 3 H), 0.89–0.85 (t, *J* = 6.9 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.38, 166.72, 155.74, 79.99, 64.26, 61.47, 47.28, 36.28, 28.25, 24.00, 16.02, 14.05, 11.60 ppm. MALDI TOF/TOF-: calcd. for $C_{15}H_{27}NO_5$ [M + Na]$^+$ 324.1787; found 324.1709.

Ethyl (*S*)-4-[(*tert*-Butoxycarbonyl)amino]-7-(methylthio)-3-oxoheptanoate (1g): Yellow viscous liquid (0.4 g, 64%). $[\alpha]_D^{25}$ = –3.75 (*c* = 0.8, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.30 (d, *J* = 8 Hz, 1 H), 4.44–4.39 (m, 1 H), 4.14 (q, *J* = 8 Hz, 2 H), 3.59–3.50 (m, 2 H), 2.49 (m, 2 H), 2.13 (m, 1 H), 2.04 (s, 3 H), 1.83–1.77 (m, 1 H), 1.39 (s, 9 H), 1.23 (t, *J* = 8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.0,167.0, 155.5, 80.3, 61.5, 60.4, 58.3, 46.3, 30.2, 30.0, 28.3, 15.4, 14.1 ppm. HRMS: calcd. for $C_{15}H_{27}NO_5S$ [M + Na]$^+$ 342.1350; found 342.1357.

Ethyl (*S*)-5-{[(Benzyloxy)carbonyl]amino}-4-[(*tert*-butoxycarbonyl)-amino]-3-oxopentanoate (1h): Yellow liquid (0.54 g, 66%). $[\alpha]_D^{25}$ = –3.00 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (s, 5 H), 5.54 (s, 1 H), 5.05 (s, 2 H), 4.41 (s, 1 H), 4.14 (q, *J* = 8 Hz, 2 H), 3.68 (d, *J* = 8 Hz, 2 H), 3.58 (s, 2 H), 3.53 (d, *J* = 8 Hz, 2 H), 1.40 (s, 9 H), 1.23 (t, *J* = 4 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.4,172.6, 167.2, 156.4,155.7, 136.0, 128.4, 128.0, 127.8, 80.2, 66.9, 62.0, 61.0, 60.4, 45.9, 39.7, 29.5, 28.1, 19.1, 13.9 ppm. HRMS: calcd. for $C_{20}H_{28}N_2O_7$ [M + Na]$^+$ 431.1794; found 431.1790.

Ethyl (*S*)-4,8-Bis[(*tert*-butoxycarbonyl)amino]-3-oxooctanoate (1i): Light yellow (0.66 g, 74%). $[\alpha]_D^{25}$ = –2.0 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.27 (m, 5 H), 5.12 (m, 2 H), 4.82 (br., 1 H), 4.18 (q, *J* = 8 Hz, 2 H), 3.50 (m, 2 H), 3.18 (m, 2 H), 1.68–1.46 (m, 8 H), 1.42 (s, 9 H), 1.26 (t, *J* = 8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 202.4, 167.0, 156.6, 136.6, 128.5, 128.1, 80.2, 77.4, 76.8, 66.73 61.6, 46.2, 40.4, 30.4, 28.5, 22.25 14.16 ppm. HRMS: calcd. for $C_{23}H_{34}N_2O_7$ [M + Na]$^+$ 473.2264; found 473.2264.

7-Benzyl (*S*)-1-Ethyl 4-[(*tert*-Butoxycarbonyl)amino]-3-oxoheptanedioate (1j): White solid (0.46 g, 57%). $[\alpha]_D^{25}$ = –2.0 (*c* = 1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.32 (m, 5 H), 5.25 (d, *J* = 8 Hz, 1 H), 5.12 (s, 2 H), 4.42 (m, 1 H), 4.18 (q, *J* = 4 Hz, 2 H), 3.57 (m, 2 H), 2.46 (m, 2 H), 2.27 (m, 1 H), 1.85 (m, 1 H), 1.43 (s, 9 H), 1.26 (t, *J* = 8 Hz, 3 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 201.8, 172.8, 166.9, 155.6, 135.8, 128.7, 80.4, 66.7, 61.7, 59.0, 46.3, 30.0, 29.8, 28.4, 26.0, 14.2 ppm. HRMS: calcd. for $C_{28}H_{29}NO_7$ [M + Na]$^+$ 430.1841; found 430.1840.

General Procedure for the Synthesis of Pyrazine Products: The appropriate Boc-γ-amino β-keto ester (1 mmol) was dissolved in CH_2Cl_2 (2 mL) and the solution was cooled in an ice bath. Then, TFA (2 mL) was added slowly to this solution. After completion of the reaction (ca. 30 min), TFA was removed from the reaction mixture under reduced pressure. The TFA salt of the γ-amino β-keto ester was dissolved in THF (5 mL) and the pH was adjusted to ca. 8 by slow addition of DIPEA (ca. 1 mL). The reaction mixture was stirred overnight in an open flask. The transformation of the γ-amino β-keto ester into the pyrazine was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude product was directly purified by column chromatography with an EtOAc/pet. ether (5%) solvent system.

Diethyl 2,2′-(3,6-Dibenzylpyrazine-2,5-diyl)diacetate (2a): White crystals (0.32 g, 79%), m.p. 95–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.27–7.14 (m, 10 H), 4.18 (s, 4 H), 4.07 (q, *J* = 7.1 Hz, 4 H), 3.81 (s, 4 H), 1.19 (t, *J* = 7.1 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.9, 151.8, 147.0, 137.9, 128.9, 128.7, 126.7, 61.2, 40.9, 40.8, 28.4, 14.2 ppm. UV: λ_{max} = 204, 281 nm. Fluorescence: λ_{ex} = 281 nm, λ_{em} = 445 nm. HRMS: calcd. for $C_{26}H_{28}N_2O_4$ [M + H]$^+$ 433.2127; found 433.2194.

Diethyl 2,2′-(3,6-Dimethylpyrazine-2,5-diyl)diacetate (2b): Colorless solid (0.174 g, 67%), m.p. 94–95 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.17 (q, *J* = 7.1 Hz, 4 H), 3.82 (s, 4 H), 2.48 (s, 6 H), 1.24 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.03, 149.54, 146.47, 61.34, 41.33, 21.20, 14.31 ppm. UV (λ_{max}) 278 nm, 211 nm. Fluorescence: λ_{ex} = 278 nm, λ_{em} = 435 nm. HRMS: calcd. for $C_{14}H_{20}N_2O_4$ [M + Na]$^+$ 281.1501; found 281.1503.

Diethyl 2,2′-(3,6-Diisobutylpyrazine-2,5-diyl)diacetate (2c): Yellow liquid (0.2 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.16 (q, *J* = 7.1 Hz, 4 H), 3.85 (s, 4 H), 2.61 (d, *J* = 7.3 Hz, 4 H), 2.17–2.10 (m, 2 H), 1.23 (t, *J* = 7.2 Hz, 6 H), 0.92 (d, *J* = 6.7 Hz, 12 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 151.6,146.2, 61.0, 42.6, 40.8, 28.2, 22.4, 14.1 ppm. UV: λ_{max} = 215 nm, 267 nm. Fluorescence: λ_{ex} = 267 nm, λ_{em} = 429 nm. HRMS (ESI): calcd. for $C_{20}H_{32}N_2O_4$ [M + H]$^+$ = 365.2240; found 365.2240.

Diethyl 2,2′-[(3,6-Diphenylpyrazine)-2,5-diyl]diacetate (2d): White solid (0.24 g, 62%), m.p. 94–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.62 (dd, *J* = 7.8, 1.7 Hz, 4 H), 7.52–7.42 (m, 6 H), 4.13 (q, *J* = 7.1 Hz, 4 H), 3.99 (s, 4 H), 1.21 (t, *J* = 7.1 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3, 151.0, 146.3, 61.2, 40.7, 33.7, 32.5, 15.8, 14.2 ppm. UV: λ_{max} = 250 nm, 292 nm. Fluorescence: λ_{ex} = 292 nm, λ_{em} = 371 nm. HRMS (ESI): calcd. for $C_{18}H_{28}N_2O_4S_2$ [M + H]$^+$ 405.1814; found 405.1815.

Diethyl 2,2′-{3,6-Bis[2-(methylthio)ethyl]pyrazine-2,5-diyl}diacetate (2g): White solid (0.252 g, 67%), m.p. 83–87. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.18 (q, *J* = 7.1 Hz, 1 H), 3.89 (s, 1 H), 3.07–3.00 (m, 1 H), 2.93–2.86 (m, 1 H), 2.12 (s, 2 H), 1.27 (t, *J* = 7.2 Hz, 2 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.3, 151.0, 146.3, 61.2, 40.7, 33.7, 32.5, 15.8, 14.1 ppm. UV: λ_{max} = 210 nm, 280 nm. Fluorescence: λ_{ex} = 280 nm, λ_{em} = 332 nm. HRMS (ESI): calcd. for $C_{18}H_{28}N_2O_4S_2$ [M + H]$^+$ 401.1568; found 401.1564.

Diethyl 2,2′-[3,6-Bis({[(benzyloxy)carbonyl]amino}methyl)pyrazine-2,5-diyl]diacetate (2h): Light yellow oil (0.36 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 (dd, *J* = 12.8, 4.3 Hz, 10 H), 6.03 (s, 4 H), 5.13 (s, 4 H), 4.52 (s, 4 H), 4.17 (q, *J* = 7.0 Hz, 4 H), 3.92 (s, 4 H), 1.26 (t, *J* = 7.1 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.5, 156.4, 148.5, 145.8, 136.4, 128.7, 128.3, 67.1, 61.7, 42.7, 40.0, 14.2 ppm. UV: λ_{max} = 216 nm, 276 nm. Fluorescence: λ_{ex} = 267 nm, λ_{em} = 441 nm. HRMS (ESI): calcd. for $C_{30}H_{34}N_4O_8$ [M + H]$^+$ 579.2455; found 579.2455; calcd. for [M + Na]$^+$ 601.2274; found 601.2287.

Diethyl 2,2′-(3,6-Bis{4-[(*tert*-butoxycarbonyl)amino]butyl}pyrazine-2,5-diyl)diacetate (2i): Pale yellow (0.52 g, 78%), m.p. 94–96 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.27 (m, 10 H), 5.07 (s, 4 H), 4.98 (s, 2 H), 4.13 (q, *J* = 7.1 Hz, 4 H), 3.81 (s, 4 H), 3.20 (q, *J* = 6.5 Hz, 4 H), 2.74 (t, *J* = 7.4 Hz, 4 H), 1.74 (p, *J* = 7.6 Hz, 4 H), 1.55 (p, *J* = 7.0 Hz, 4 H), 1.21 (t, *J* = 7.1 Hz, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.2, 146.1, 136.7, 128.1, 128.1, 66.6, 61.3, 40.7, 33.1, 29.3, 25.2, 14.2 ppm. UV: λ_{max} = 278 nm. Fluorescence: λ_{ex} = 278 nm, λ_{em} = 319 nm. HRMS: calcd. for $C_{36}H_{46}N_4O_8$ [M + H]$^+$ 663.3388; found 663.3384.

Dibenzyl 3,3′-[3,6-Bis(2-ethoxy-2-oxoethyl)pyrazine-2,5-diyl]dipropanoate (2j): White solid (0.343 g, 60%), m.p. 95–97 °C. ^{1}H NMR

(400 MHz, $CDCl_3$): δ = 7.55–7.12 (m, 10 H), 5.09 (s, 4 H), 4.13 (q, J = 7.1 Hz, 1 H), 3.80 (s, 4 H), 3.04 (t, J = 7.1 Hz, 4 H), 2.87 (t, J = 7.2 Hz, 4 H), 1.22 (t, J = 7.1 Hz, 6 H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$): δ = 201.80, 172.79, 166.93, 135.74, 128.38, 80.41, 66.69, 61.68, 60.50, 58.99, 46.25, 30.04, 28.36, 26.00, 14.15 ppm. UV: λ_{max} = 208 nm, 278 nm. Fluorescence: λ_{ex} = 278 nm, λ_{em} = 337 nm. HRMS: calcd. for $C_{32}H_{36}N_2O_8$ $[M + H]^+$ 577.2550; found 577.2532.

Synthesis of Diethyl 2,2′-[3,6-Bis({[(benzyloxy)amino]-3-methylbutanamido}methyl)pyrazine-2,5-diyl]diacetate (C1): Cbz-Val-Dap(Boc)-CHO was dissolved in CH_2Cl_2 (10 mL) at room temperature, and then tin(II) chloride (0.059 g, 20 mol-%) was added, followed by ethyl diazoacetate (0.152 g, 1.3 mmol). Immediate gas evolution was observed. The reaction mixture was stirred and the progress of the reaction was monitored by TLC. After completion of the reaction (ca. 30 min), the mixture was quenched with HCl (0.5 N, 5 mL) and then extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was washed with brine (20 mL), dried with anhydrous sodium sulfate, and concentrated under reduced pressure to afford a greenish oily crude product. This was purified by silica gel column chromatography to afford pure peptide **P2** (0.336 g, 60%), which was directly used for the pyrazine synthesis. Peptide **P2** (0.4 g, 0.8 mmol) was dissolved in CH_2Cl_2 (2 mL) and the solution was cooled in an ice bath. Then TFA (2 mL) was added slowly to this solution. After completion of the reaction (ca. 30 min), TFA was removed from the reaction mixture under reduced pressure. The TFA salt **P3** was dissolved in THF (5 mL), and the pH was adjusted to ca. 8 by slow addition of DIPEA (ca. 1 mL). The reaction mixture was stirred overnight in an open flask. The transformation of the γ-amino β-keto ester into the pyrazine was monitored by TLC. After completion of the reaction, the solvent was evaporated under reduced pressure, and the crude product was directly subjected to column chromatography and purified with an EtOAc/pet. ether (35%) solvent system to afford pure **C1** (0.15 g) in 55% yield. Light yellow solid (0.17 g). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.34 (s, 10 H), 7.18 (s, 2 H), 5.43 (s, 2 H), 5.10 (d, J = 4 Hz, 4 H), 4.57 (s, 4 H), 4.24–4.15 (q, J = 8 Hz, 4 H), 4.10 (m, 2 H), 3.92 (*s*, 4 H), 2.14 (m, 2 H), 1.27 (t, J = 8 Hz, 6 H), 0.93 (dd, J = 16.0, 8 Hz, 12 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 169.5, 156.5, 148.4, 145.9, 136.3, 128.7, 128.3, 128.2, 67.2, 61.8, 60.5, 41.0, 40.0, 31.4, 25.7, 19.3, 17.9, 14.3 ppm. UV: λ_{max} = 208 nm, 278 nm. Fluorescence: λ_{ex} = 276 nm, λ_{em} = 314 nm. MALDI-TOF/TOF: calcd. for $C_{14}H_{52}N_6O_{10}$ $[M + H]^+$ 777.3823; found 777.3875; calcd. for $[M + Na]^+$ 799.3643; found 799.3701.

Diethyl 2,2′-{3,6-Bis[(5*S*,8*S*)-5-isobutyl-8-methyl-3,6,9-trioxo-1-phenyl-2-oxa-4,7,10-triazaundecan-11-yl]pyrazine-2,5-diyl}diacetate (C2): Cbz-Leu-Ala-Dap(Boc)-CHO was dissolved in CH_2Cl_2 (15 mL) at room temperature, and then tin(II) chloride (0.139 g, 20 mol-%) was added, followed by ethyl diazoacetate (0.353 g, 3 mmol). Immediate gas evolution was observed. The reaction mixture was stirred and the progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was quenched with HCl (0.5 N, 5 mL) and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layer was washed with brine (20 mL), dried with anhydrous sodium sulfate, and concentrated under reduced pressure to afford a greenish oily crude product. This was purified by silica gel column chromatography to afford pure peptide **P5** (0.71 g, 40%). Peptide **P5** (0.3 g, 0.5 mmol) was dissolved in CH_2Cl_2 (2 mL) and the solution was cooled in an ice bath. Then TFA (2 mL) was added slowly to this solution. After completion of the reaction (ca. 30 min), TFA was removed from the reaction mixture under reduced pressure. The TFA salt **P6** was dissolved in THF (5 mL), and the pH was adjusted to ca. 8 by slow addition of DIPEA (ca. 1 mL). The reaction mixture was stirred in an open flask. The transformation of the tripeptide γ-amino β-keto ester into the pyrazine was monitored by MALDI-TOF/TOF. After completion of the reaction, the solvent was evaporated under reduced pressure and the crude product was then purified by reversed-phase HPLC to afford the desired pyrazine **C2** (0.12 g) in 50% yield. Light yellow solid (0.12 g). Retention time (t_R) = 22 min (mobile phase $MeOH/H_2O$). ^1H NMR (400 MHz, $CDCl_3$): δ = 7.75–7.22 (m, 14 H), 6.06 (s, 2 H), 5.25–5.01 (m, 6 H), 4.55 (s, 4 H), 4.21–4.16 (m, 3 H), 3.93 (s, 4 H), 1.64–1.20 (m, 16 H), 1.67 (s, 8 H), 1.39 (s, 4 H), 1.39 (s, 4 H), 1.28 (m, 6 H), 0.93 (m, 12 H) ppm. UV: λ_{max} = 278 nm. Fluorescence: λ_{ex} = 280 nm, λ_{em} = 320 nm. MALDI-TOF/TOF: calcd. for $C_{48}H_{66}N_8O_{12}$ $[M + Na]^+$ 969.4689; found 969.6588.

Supporting Information (see footnote on the first page of this article): ORTEP diagrams of compounds **2a** and **2b**, copies of ^1H and ^{13}C NMR and mass spectra for all synthesized compounds and RP-HPLC profile of pyrazine **C2** are available.

Acknowledgments

The authors thank Indian Institute of Science Education and Research (IISER-Pune) for financial support. M. G. K. is grateful to the Council of Scientific and Industrial Research (CSIR), New Delhi for a Senior Research Fellowship.

[1] a) G. R. Pettit, B. R. Moser, R. F. Mendonça, J. C. Knight, F. J. Hogan, *Nat. Prod.* **2012**, *5*, 1063; b) B. R. Moser, *J. Nat. Prod.* **2008**, *71*, 487; c) S. Lee, T. G. LaCour, P. L. Fuchs, *Chem. Rev.* **2009**, *109*, 2275; d) A. K. Kumar, J. J. La Clair, P. L. Fuchs, *Org. Lett.* **2011**, *13*, 5334.
[2] J. A. Walker, W. Liu, D. S. Wise, J. C. Drach, L. B. Townsend, *J. Med. Chem.* **1998**, *41*, 1236.
[3] a) H. I. Boshoff, V. Mizrahi, C. E. Barry, *J. Bacteriol.* **2002**, *184*, 2167; b) O. Zimhony, J. S. Cox, J. T. Welch, C. Vilchèze, W. R. Jacobs Jr., *Nat. Med.* **2000**, *6*, 1043.
[4] a) B. Bohman, L. Jeffares, G. Flematti, L. T. Byrne, B. W. Skelton, R. D. Phillips, K. W. Dixon, R. Peakall, R. A. Barrow, *J. Nat. Prod.* **2012**, *75*, 1589; b) B. Bohman, L. Jeffares, G. Flematti, R. D. Phillips, K. W. Dixon, R. Peakall, R. A. Barrow, *Org. Lett.* **2012**, *14*, 2576; c) B. Bohman, R. D. Phillips, M. H. M. Menz, B. W. Berntsson, G. R. Flematti, R. A. Barrow, K. W. Dixon, R. R. Peakall, *New Phytol.* **2014**, *203*, 939.
[5] J. W. Wheeler, M. S. Blum, *Science* **1973**, *182*, 501.
[6] S. Rappert, K. C. Botsch, S. Nagorny, W. Francke, R. Muller, *Appl. Environ. Microbiol.* **2006**, *72*, 1437.
[7] T. B. Adams, J. Doull, V. J. Feron, J. I. Goodman, L. J. Marnett, I. C. Munro, P. M. Newberne, P. S. Portoghese, R. L. Smith, W. J. Waddell, B. M. Wagner, *Food Chem. Toxicol.* **2002**, *40*, 429.
[8] a) H.-Y. Liu, H. Wu, J.-F. Ma, J. Yang, Y.-Y. Liu, *Dalton Trans.* **2009**, *38*, 7957; b) R. Mondal, S. Ko, Z. Bao, *J. Mater. Chem.* **2010**, *20*, 10568; c) R. J. Crutchley, *Angew. Chem. Int. Ed.* **2005**, *44*, 6452; *Angew. Chem.* **2005**, *117*, 6610; d) X. Lu, S. Fan, J. Wu, X. Jia, Z.-S. Wang, G. Zhou, *J. Org. Chem.* **2014**, *79*, 6480.
[9] T. Kosuge, T. Adachi, H. Kamiya, *Nature* **1962**, *195*, 1103.
[10] a) L. Chill, M. Aknin, Y. Kashman, *Org. Lett.* **2003**, *5*, 2433; b) M. Peña-López, M. M. Martínez, L. A. Sarandeses, J. Pérez Sestelo, *Org. Lett.* **2010**, *12*, 852.
[11] a) W. Yong, J. B. Gloer, J. A. Scott, D. Malloch, *J. Nat. Prod.* **1995**, *58*, 93; b) K. Tatsuka, K. Fujimoto, M. Yamashita, T. Tsuchiya, S. Umeyawa, H. Umeyawa, *J. Antibiot.* **1973**, *26*, 606; c) M. Devys, M. Barbier, A. Kollmann, J.-F. Bousquet, *Tetrahedron Lett.* **1982**, *23*, 5409; d) J. S. Dickschat, H. Reichenbach, I. Wagner-Döbler, S. Schulz, *Eur. J. Org. Chem.* **2005**, 4141; e) S. Schulz, J. Fuhlendorff, H. Reichenbach, *Tetrahedron* **2004**,

60, 3863; f) M. Shaaban, R. P. Maskey, I. Wagner-Döbler, H. Laatsch, *J. Nat. Prod.* **2002**, *65*, 1660; g) S.-X. Huang, E. Powell, S. R. Rajski, L.-X. Zhao, C.-L. Jiang, Y. Duan, W. Xu, B. Shen, *Org. Lett.* **2010**, *12*, 3525.

[12] S. Kutanovas, J. Stankeviciute, G. Urbelis, D. Tauraite, R. Rutkiene, R. Meskysa, *Appl. Environ. Microbiol.* **2013**, *79*, 3649.

[13] a) R. M. Adlington, J. E. Baldwin, D. Catterick, G. J. Pritchard, *J. Chem. Soc. Perkin Trans. 1* **2001**, *1*, 668; b) M. L. H. Mantel, A. T. Lindhardt, D. Lupp, T. Skrydstrup, *Chem. Eur. J.* **2010**, *16*, 5437; c) J.-T. Hou, Y. H. Liu, Z. H. Zhang, *J. Heterocycl. Chem.* **2010**, *47*, 703; d) Z. Zhao, D. D. Wisnoski, S. E. Wolkenberg, W. H. Leister, Y. Wang, C. W. Lindsley, *Tetrahedron Lett.* **2004**, *45*, 4873; e) G. B. Barlin, *The Chemistry of Heterocyclic Compounds*, Wiley, New York, **1982**, vol. 41.

[14] F. Buron, A. Turck, N. Ple, L. Bischoff, F. Marsais, *Tetrahedron Lett.* **2007**, *48*, 4327.

[15] T. Utsukihara, H. Nakamura, M. Watanabe, A. C. Horiuchi, *Tetrahedron Lett.* **2006**, *47*, 9359.

[16] Z. Chen, D. Ye, G. Xu, M. Ye, L. Liu, *Org. Biomol. Chem.* **2013**, *11*, 6699.

[17] A. Vidal-Albalat, S. Rodriguez, F. V. Gonzalez, *Org. Lett.* **2014**, *16*, 1752.

[18] B. Gnanaprakasam, E. Balaraman, Y. Ben-David, D. Milstein, *Angew. Chem. Int. Ed.* **2011**, *50*, 12240.

[19] F. Palacios, A. M. Ochoa de Retana, J. I. Gil, R. Lopez de Munain, *Org. Lett.* **2002**, *4*, 2405.

[20] D. F. Taber, P. W. DeMatteo, K. V. Taluskie, *J. Org. Chem.* **2007**, *72*, 1492.

[21] S. Badrinarayanan, J. Sperry, *Org. Biomol. Chem.* **2012**, *10*, 2126.

[22] N. Rojas, Y. Grillasca, A. Acosta, I. Audelo, G. G. de la Mora, *J. Heterocycl. Chem.* **2013**, *50*, 982.

[23] G. R. Pettit, T. H. Smith, S. Feng, J. C. Knight, R. Tan, R. K. Pettit, P. A. Hinrichs, *J. Nat. Prod.* **2007**, *70*, 1073.

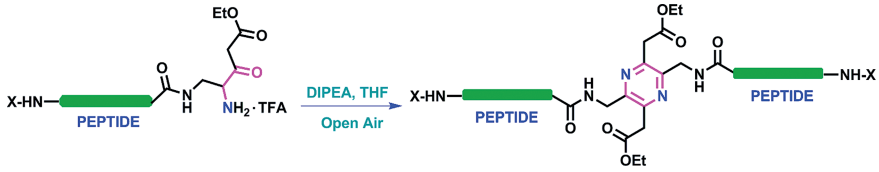
[24] a) A. Bandyopadhyay, N. Agarwal, S. M. Mali, S. V. Jadhav, H. N. Gopi, *Org. Biomol. Chem.* **2010**, *8*, 4855; b) A. Bandyopadhyay, A. Malik, M. Ganesh Kumar, H. N. Gopi, *Org. Lett.* **2014**, *16*, 294; c) A. Bandyopadhyay, H. N. Gopi, *Org. Biomol. Chem.* **2011**, *9*, 8089.

[25] C. R. Holmquist, E. J. Roskamp, *J. Org. Chem.* **1989**, *54*, 3258.

[26] a) G. R. Pettit, M. Inoue, D. L. Herald, T. S. Krupa, *J. Am. Chem. Soc.* **1988**, *110*, 2006; b) S. Fukuzawa, S. Matsunaga, N. Fusetani, *J. Org. Chem.* **1994**, *59*, 6164.

Received: September 19, 2014
Published Online: ■

Peptide Self-Dimerization



A facile synthesis of highly symmetrical tetrasubstituted pyrazines through simple aerial oxidation of β-keto γ-esters derived from amino acids and peptides is reported.

M. Ghanesh Kumar, V. J. Thombare, R. D. Bhaisare, A. Adak, H. N. Gopi* **1–8**

Synthesis of Tetrasubstituted Symmetrical Pyrazines from β-Keto γ-Amino Esters: A Mild Strategy for Self-Dimerization of Peptides

Keywords: Amino acids / Peptides / Oxidation / Cyclization / Nitrogen heterocycles

