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Diacetylenic isobutylamides of *Echinacea*: synthesis and natural distribution

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Abstract

The syntheses of three diacetylenic isobutylamides of *Echinacea angustifolia* have been achieved by direct synthetic routes by way of a common intermediate. The key step is the alkylation of the anion of the silylated diacetylene. We report the presence of all three diacetylenic isobutylamides in six of the nine *Echinacea* species: *E. angustifolia*, *E. sanguinea*, *E. simulata*, *E. tennesseensis*, *E. atrorubens and E. laevigata*. The accumulation of these amides is sensitive to organ type and age.

Keywords: *Echinacea*; Asteraceae; Synthesis; Diacetylenic isobutylamides; Alkamides; Alkylamides

1. Introduction

Polyacetylenes are a class of natural products found mainly in plants. The pioneering studies of both Bohlmann (1988) and Bauer (2000) have revealed much useful information about the structure, chemistry, and biological activity of this novel class of compounds. Acetylenes are found in each of the commonly used species of *Echinacea*. Amide **1** is a significant constituent of *E. purpurea* (Bauer, 2000), whereas ketone **2** is found mainly in *E. pallida* (Binns et al., 2002), and diacetylenic amides **3–5** are found in *E. angustifolia* (Bauer et al., 1989) (Fig. 1). A complex mixture containing at least 12 different acetylenic amides can be obtained by supercritical fluid extraction of fresh dried roots of *E. angustifolia* (Sun et al., 2002). Crombie and co-workers have reported elegant syntheses of natural amides related to **1–4** using organometallic coupling reactions (Crombie and Harper, 1949; Crombie and Manzoor-I-Khuda, 1957; Crombie and Fisher, 1985; Crombie et al., 1987). Wailes (1959) has also reported the syntheses of natural dienamides. These amides have since been shown to be active against *Aedes aegyptii* larvae and *Helicoverpa zea* neonates at the microgram per milliliter level (Ramsewak et al., 1999). Authentic standards of amides such as **3**, **4**, and **5** are important for both plant metabolomic studies and for structure–activity studies to determine the bioactivities of these compounds in heterologous species.

In conjunction with a broad-based effort in the study of metabolites of *Echinacea* and *St. John's wort* (Kraus and Bae, 2003), we report the first syntheses of three naturally occurring amides by direct and flexible synthetic routes. The distribution of these diacetylenic isobutylamides in accessions of the nine species of *Echinacea* (*E. angustifolia*, *E. purpurea*, *E. pallida*, *E. sanguinea*,

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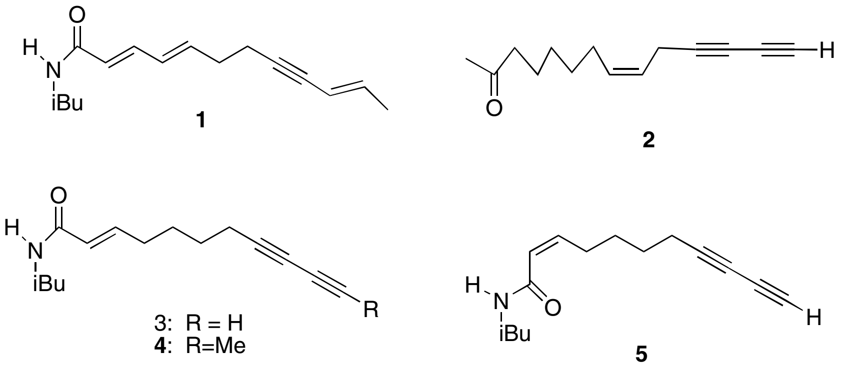


Fig. 1. Typical acetylenes found in *Echinacea* species: (*E*,*Z*,*E*)-*N*-isobutyl dodeca-2,4,10-triene-8-ynamide (**1**), (*Z*)-tetradeca-8-ene-11,13-diyn-2-one (**2**), (*E*)-*N*-isobutyl undeca-2-ene-8,10-diynamide **(3),** (*E*)-*N*-isobutyl dodeca-2-ene-8,10-diynamide **(4),** (*Z*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**5**).

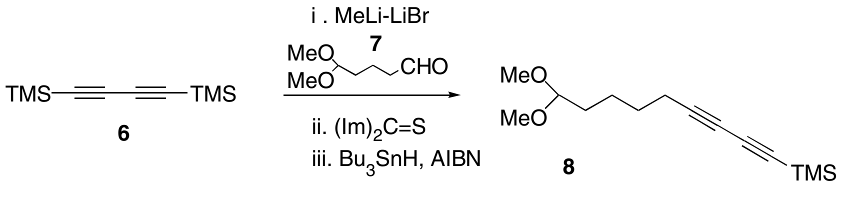
E. simulata, *E. tennesseensis*, *E. atrorubens*, *E. laevigata* and *E. paradoxa*) is described.

2. Results and discussion

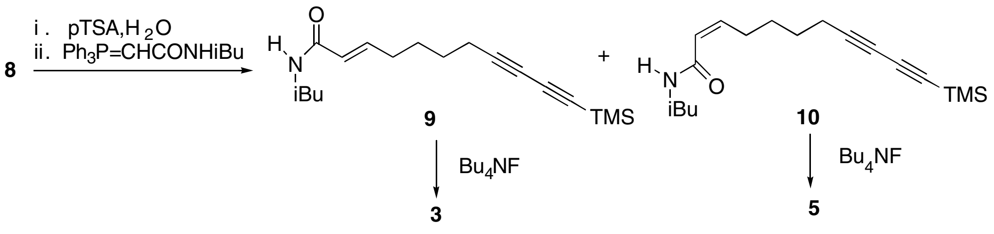
2.1. Synthesis of diacetylenic isobutylamides

Our route began with the construction of acetal **8** (Scheme 1) from commercially available bis-trimethylsilyldiacetylene **6** and aldehyde **7** that was readily available from the ozonolysis of cyclopentene by the method of Schreiber (Schreiber et al., 1982). Generation of the monoanion from **6** with methyl lithium–lithium bromide complex in THF at 0 °C followed by reaction at −78 °C with aldehyde **7** afforded a propargylic alcohol in 88% isolated yield (Holmes and Jones, 1980). Deoxygenation was achieved by reaction of the alcohol with thiocarbonyldiimidazole (CH_2Cl_2, 25 °C) followed by treatment with two equivalents of tributyltin hydride and AIBN at 80 °C in toluene to produce acetal **8** in 51% yield over two steps (Gunji and Vasella, 2000). The use of larger quantities of tributyltin hydride should be avoided since addition to the acetylene occurred. Although alkylation of the anion of the diacetylene would have been more direct, several attempts to effect the direct alkylation provided only low yields of the desired product.

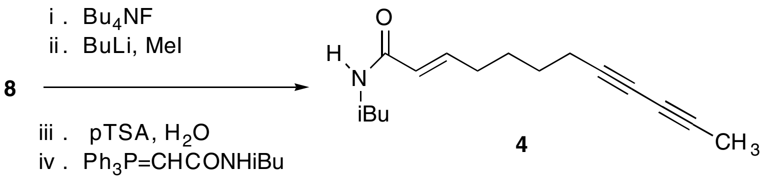
Acetal **8** was the key intermediate for the syntheses of amides **3**–**5** (Scheme 2). Hydrolysis of the acetal using pTSA in aqueous acetone at ambient temperature gave almost a quantitative yield of aldehyde. The aldehyde reacted with the amide phosphorane to afford *E*-isomer **9** in 73% yield (Barrett et al., 1996). Approximately 10%



Scheme 1. Synthesis of acetal **8** from bis-trimethylsilyldiacetylene **6** and aldehyde **7**.



Scheme 2. Synthesis of diacetylenic isobutylamides **3** and **5** from acetal **8**.



Scheme 3. Synthesis of diacetylenic isobutylamide **4** from acetal **8**.

of the *Z*-isomer **10** was also formed and was readily separable from the *E*-isomer by silica gel flash chromatography. The reaction of *E*-amide **9** with tetrabutylammonium fluoride (TBAF) in THF at 0 °C produced **3** in 95% yield. The reaction of *Z*-isomer **10** generated compound **5** in 97% yield.

The synthesis of **4** from **8** (Scheme3) began with a desilylation reaction using TBAF followed by methylation of the resulting terminal acetylene with *n*-butyl lithium and methyl iodide. The latter reaction was very slow and worked better when an equivalent of hexamethylphosphoric triamide was added after generation of the acetylide anion. Hydrolysis of the acetal followed by Wittig reaction afforded diacetylene **4** in 46% yield. The proton and carbon NMR spectra of the synthesized amides **3-5** were identical to the spectra reported by Ramsewack (Ramsewak et al., 1999), and both their HPLC retention volumes and UV spectra were identical to those from natural materials (Fig. 2(a)). The three amides were well separated by the solvent system employed. Their elution sequence on reversed phase C18 column was influenced by the chain length and the stereochemistry of the double bond at carbon 2, with amides **3, 5** and **4** eluted at 17.6 min, 20.2 min and 21.2 min successively. The three amides demonstrated very similar UV spectra, all with an absorption maximum at 210 nm caused by the α,β unsaturated amide chromophore, which agrees with that reported by Bauer and Remiger (1989).

The identity of amides **3**–**5** was further confirmed by comparison of their retention times and mass spectra using GC–MS (Fig. 2(b)). They were well separated by GC, with amide **5** eluting at 23.1 min followed by amide **3** and **4** at 25.1 min and 28.5 min, respectively. EIMS analysis afforded characteristic mass spectrum for each of the amides, i.e. amides **3**, **4** and **5** gave molecular ions at *m*/*z* 231, 245 and 231, which were calculated for $C_{15}H_{21}ON$, $C_{16}H_{23}ON$ and $C_{15}H_{21}ON$, respectively. Molecular ion and many fragment ions of amide **4** were 14 mass units greater than those of amide **3** and **5** due to the terminal methyl group. The mass spectra of amide **3** and **5** are very similar but can be distinguished by relative abundance of some of the fragment ions, eg. fragments $m/z = 131$ ($[M - C_5H_{10}NO]^+$) and $m/z = 91$ ($[C_7H_7]^+$). Both GC retention times and mass spectra for the synthetic compounds were identical to those from the natural materials.

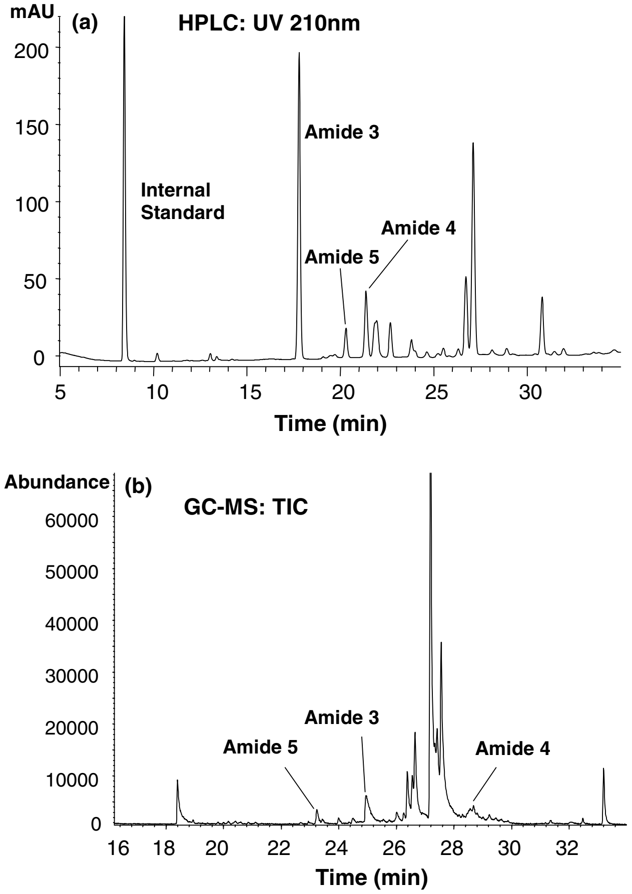


Fig. 2. HPLC chromatogram (a) and GC–MS TIC chromatogram (b) obtained from a 95% ethanolic extract of 3-month-old *E. angustifolia* roots, indicating diacetylenic isobutylamides **3**–**5**.

2.2. *Characterization and distribution of the diacetylenic isobutylamides in Echinacea*

Monoene-type isobutylamides **3**–**5** were first reported in *E. angustifolia* roots (Bauer et al., 1989) and later were identified in roots of *E. tennesseensis* (Bauer et al., 1990) and *E. simulata* (Bauer and Foster, 1991). They are often used as one of the diagnostic patterns for identification of *E. angustifolia* roots in commercial preparations (Bauer, 1998). Binns et al. (2002) have reported that diacetylenic isobutylamides are present to some extent in all *Echinacea* species, however, in that report HPLC peaks were identified as diacetylenic isobutylamides based solely on their UV spectra and their

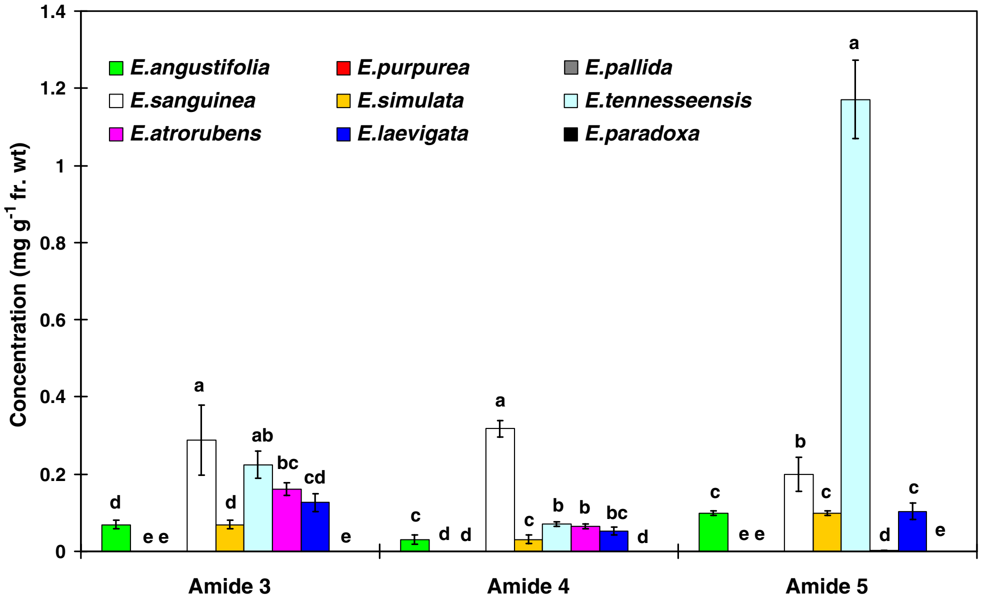


Fig. 3. Concentration of diacetylenic isobutylamides **3**–**5** in 6-month-old roots of nine species of *Echinacea.* Error bars indicate standard deviations of means of triplicate experiments. For each diacetylenic isobutylamide (**3**, **4** or **5**), different letters indicate a significant difference ($p<0.05$).

retention times as compared to those of non-diacetylenic isobutylamide standards.

Using the authentic diacetylenic isobutylamides **3**–**5**, we combined GC–MS and HPLC to identify and quantify their distribution in plants. The mean levels of amides **3**–**5** in roots from 6-month-old plants from nine *Echinacea* species are presented in Fig. 3. Our results show that of the 9 *Echinacea* species, compounds **3**–**5** present in six species: *E. angustifolia*, *E. sanguinea*, *E. simulata*, *E. tennesseensis*, *E. atrorubens* and *E. laevigata.* Among these six species, the highest concentrations of amide **3** (0.29 ± 0.02 mg g^{-1} fr. wt) and amide **4** (0.32 ± 0.03 mg g^{-1} fr. wt) are found in *E. sanguinea*, whereas *E. tennesseensis* contains the highest amount of amide **5** (1.2 ± 0.1 mg g^{-1} fr. wt). Studies of the distributions of the three amides in different organs of *E. angustifolia* indicate they are present mainly in roots, have reduced abundance in flowers, and are not detectable (the limit of HPLC detection for all 3 amides is approximately 0.02 μg ml^{-1}) in leaves (Fig. 4). Moreover, the ratio of amides **3**–**5** in *E. angustifolia* roots changes with development. In 3-month-old roots, the ratio of amide **3** to its isomer amide **5** was 1:9, whereas in 6-month-old roots this ratio was about 2:3, a difference significant at $p<0.01$. This indicates that isomerisation of the 2-monoene portion of these amides may be regulated during root growth and development.

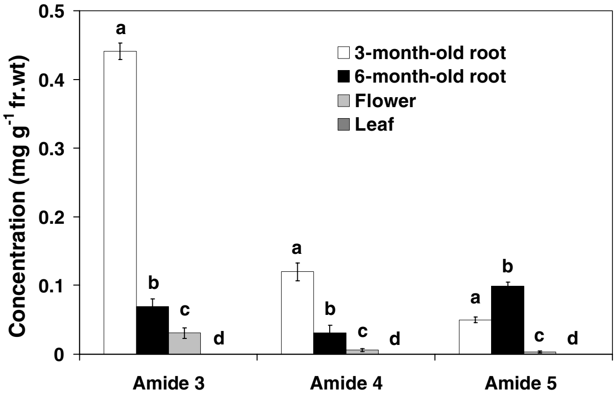


Fig. 4. Concentration of diacetylenic isobutylamides **3**–**5** in *E. angustifolia* roots from 3 and 6-month-old plants, and flowers, and leaves of 6-month-old plants. Error bars indicate standard deviations of means of triplicate experiments. For each diacetylenic isobutylamide (**3**, **4** or **5**), different letters indicate a significant difference ($p<0.05$).

3. Concluding remarks

Diacetylenic isobutylamides **3**–**5** have been synthesized in eight steps from cyclopentene by direct and flexible synthetic routes. The presence of these amides in *Echinacea* has been confirmed by comparison of retention times, UV spectra and mass spectra using HPLC and GC–MS. They are distributed widely in *Echinacea*, being present in at least six of the nine species (*E. angustifolia*, *E. sanguinea*, *E. simulata*, *E. tennesseensis*, *E. atrorubens* and *E. laevigata)* examined. The abundance of these compounds varies with organ type and plant age. Extension of this work to the syntheses of other members of this class of amides and their natural distribution will further define the metabolic variation among and within *Echinacea* species.

4. Experimental

4.1. General analytical procedures

Tetrahydrofuran and Et_2O were distilled from sodium benzophenone ketyl, whereas CH_2Cl_2, C_6H_6 and diisopropylamide were distilled over calcium hydride. All experiments were performed under argon atmosphere unless otherwise noted. Organic extracts were dried over anhydrous $MgSO_4$. Infrared spectra were obtained on a Perkin–Elmer model 1320 spectrophotometer. Nuclear magnetic resonance experiments were performed with either a Varian 300 MHz or Bruker 400 MHz instrument. High resolution mass spectra were recorded on a Kratos model MS-50 spectrometer and low resolution mass spectra were performed with a Finnegan 4023 mass spectrometer. Sgc is silica gel flash column chromatography.

4.2. Plant material and extraction

Seedlings of *E. angustifolia* (Accession 631267), *E. purpurea* (Accession 631307), *E. pallida* (Accession 631293), *E. sanguinea* (Accession A23878), *E. simulata* (Accession 631249), *E. tennesseensis* (Accession 631325), *E. atrorubens* (Accession 631262), *E. laevigata* (Accession 631312) *and E. paradoxa* (Accession 631301) were provided by Dr. Mark P. Widrlechner at the USDA-ARS North Central Regional Plant Introduction Station. Plants were grown under ambient light in a greenhouse in 7.5 inch pots in soil (50% Canadian Peat Moss + 40% Perlite + 10% mineral soil), at 22–25 °C, with daily watering. Pots were laid out in a completely randomized design generated from random digits table (Clarke and Kempson, 1997) on a single greenhouse bench. Plant samples were harvested from 3-month-old and 6-month-old plants between 9 and 10 a.m, to standardize plant material with respect to possible diurnal variations in isobutylamide content. Specifically, we used: recently mature leaves expanded to over 90% of their final length and located in the mid section of stems; flowers with 75–90% of the disc florets open, fully expanded ligulate florets and intensive pollen grain release; and entire roots. After harvest, plant material was immediately ground to a fine powder in liquid N_2 in a mortar and pestle and stored in liquid N_2 until extraction. An aliquot of this powdered plant material (0.3 g fr. wt per sample) was further ground in liquid N_2 in a mortar and pestle with addition of 25 μl (1 mg ml^{-1}) 7-hydroxy-(*E*)-*N*-isobutylundeca-2-ene-8,10-diynamide ($C_{15}H_{21}O_2$) as an internal standard, then with 1 ml of 95% ethanol for 2 min, and the resultant suspension was transferred to a capped tube. 95% ethanol (2 ml) was used to rinse the mortar and this was added to the suspension. The tube was vortexed 30 sec, and centrifuged 1 min at 12,000*g*. The supernatant (hence referred to as ethanol extract) was filtered through a 0.22 μm PTFE filter (Alltech, IL). All experiments were performed in triplicate on independently extracted plant samples from three individual plants.

4.3. HPLC analysis

Ethanol extract (15 μl) was injected into a Beckman Coulter HPLC with a 508 autosampler, 126 pump control and 168 UV-photodiode array detector (PDA) controlled by 32karat ^{TM} software (Version 5.0), and a YMC-Pack ODS-AM RP C18 (250×4.6 mm, 5 μm) column (Waters, MA). The solvent system used was CH_3CN/H_2O at a flow rate of 1.0 ml/min following a linear gradient of 40–80% CH_3CN in H_2O over 45 min. Online UV spectra were collected between 200–400 nm. Amides were quantified based on the internal standard because they have the same UV absorption at 210 nm. The limit of HPLC detection for all three amides is approximately 0.02 μg ml^{-1}.

4.4. GC–MS analysis

GC–MS analyses were performed with a GC series 6890 from Agilent (Palo Alto, CA) coupled with a 5973 Agilent mass detector operating in the EI mode (70 eV), using a HP- 1 silica capillary column (30 m×0.32 mm i.d.; film thickness 0.25 μm), and helium as the carrier gas. Extracts were evaporated to dryness under N_2 and resuspended in chloroform (2 ml). The chloroform-dissolved sample (1 μl) was injected using splitless injection mode. The temperature of both the injector and detector was at 250 °C. The column temperature was programmed to increase after 2 min from 80 to 260 °C at a rate of 5 °C/min, then held for 10 min, up to 320 °C at a rate of 5 °C/min, then held for 5 min. Resulting chromatograms were integrated by Agilent's HP enhanced ChemStation TM G1701 BA version B.01.00 software. Peaks were identified by their mass spectra and retention times.

4.5. Statistical analysis

Statistical analyses were performed using SAS software version 8.02 (SAS Institute Inc., Cary, NC). One-way analysis of variance followed by the Tukey test was used to compare means. Significance of difference was defined at $p < 0.05$.

4.6. Synthesis of amides

4.6.1. 9,9-Dimethoxy-1-trimethylsilyl-1,3-nonadiyne (**8**)

To a solution of 1,4-bistrimethylsilylbutadiyne (1.63 g, 8.4 mmol) in 10 ml of THF was added 1.5 M MeLi–LiBr (1.5 M solution, 5.58 ml) at 0 °C. The mixture was warmed up to rt. After stirring for 3 h at rt the mixture was cooled to −78 °C. To the mixture was added aldehyde **7** (0.50 g, 3.4 mmol) in THF. After stirring for 45 min at −78 °C, water (50 ml) was added. The mixture was then extracted with ether (50 ml), washed with brine, and dried (MgSO$_4$). The residue was purified by sgc (hexane:ethyl acetate = 4:1) to give the product (0.793 g, 88%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 4.43 (1H, *q*, $J=5.1$ Hz), 4.37 (1H, *t*, $J=5.7$ Hz), 3.32 (6H, *s*), 1.87 (1H, br *s*), 1.71–1.78 (2H, *m*), 1.58–1.68 (2H, *m*), 1.48–1.56 (4H, *m*), 0.19 (9H, *s*); ^{13}C NMR (75 MHz, CDCl$_3$): δ 104.5, 87.6, 87.5, 78.9, 69.9, 62.6, 52.9, 37.3, 32.2, 20.4, −0.29.

To a solution of the compound produced above (0.71 g, 2.65 mmol) in CH$_2$Cl$_2$ (10 ml) was added 1,1-thiocarbonyldiimidazole (0.94 g, 5.3 mmol) at rt. After stirring for 12 h, the solvent was removed in vacuo. The residue was purified by sgc (hexane:ethyl acetate = 3:1) to give the product (0.86 g, 86%). ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.27 (1H, *t*, $J=0.9$ Hz), 7.56 (1H, *t*, J = 1.5 Hz), 6.98 (1H, *q*, $J=0.9$ Hz), 5.99 (1H, *t*, $J=6.6$ Hz), 4.33 (1H, *t*, $J=5.1$ Hz), 3.27 (6H, *s*), 1.98–2.01 (2H, *m*), 1.55–1.64 (4H, *m*), 0.15 (9H, *s*).

To a solution of the thioimidazolide produced above (0.91 g, 2.4 mmol) in toluene was added AIBN (0.039 g, 0.24 mmol) and Bu$_3$SnH (0.71 ml, 2.64 mmol) at rt. The mixture was boiled at 80 °C for 1 h. It was cooled to rt and solvent was removed in vacuo. The residue was purified by sgc (hexane:ethyl acetate = 10:1) to give the acetal **8** (0.42 g, 69%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.34 (1H, *t*, $J=5.6$ Hz), 3.30 (6H, *s*), 2.27 (1H, *t*, $J=6.8$ Hz), 1.46–1.61 (4H, *m*), 1.39–1.45 (2H, *m*), 0.18 (9H, *s*); ^{13}C NMR (100 MHz, CDCl$_3$): δ 104.5, 88.6, 83.3, 79.9, 65.9, 52.9, 32.2, 28.1, 24.1, 19.4, −0.12; HREIMS [M]$^+$ m/z: 252.1549 (Calc. 252.1546) for C$_{14}$H$_{24}$O$_2$Si.

4.6.2. (*E*)-*N*-isobutyl 11-trimethylsilyl-undeca-2-ene-8,10-diynamide (**9**) and (*Z*)-*N*-isobutyl 11-trimethylsilyl-undeca-2-ene-8,10-diynamide (**10**)

To a solution of acetal **8** (0.11 g, 0.44 mmol) in Me$_2$CO/H$_2$O (5 ml/0.5 ml) was added pTSA (0.009 g, 0.044 mmol) at rt. After stirring for 12 h at rt, the solvent was removed. Water (25 ml) was added and the mixture was extracted with ether (50 ml), washed with sat NaHCO$_3$, brine, and dried (MgSO$_4$). The residue was purified by sgc (hexane:ethyl acetate = 4:1) to give an aldehyde that was taken on to the next step (0.081 g, 89%). IR $\nu_{\max}$ (neat) cm^{-1}: 2958, 2359, 2225, 2108, 1708, 1250, 846; ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.75 (1H, *t*, $J=1.8$ Hz), 2.45 (2H, *td*, $J=6.9$, 1.8 Hz), 2.30 (2H, *t*, $J=6.9$ Hz), 1.68–1.78 (2H, *m*), 1.50–1.60 (2H, *m*), 0.14 (9H, *s*); ^{13}C NMR (75 MHz, CDCl$_3$): δ 202.1, 88.5, 83.7, 79.3, 66.2, 43.4, 27.7, 21.4, 19.3, −0.1; HREIMS [M]$^+$ m/z: 206.1130 (Calc. 206.1127) for C$_{12}$H$_{18}$OSi.

To a solution of triphenyl-(*N*-isobutylcarboxamidomethyl)-phosphonium bromide (0.415 g, 1.01 mmol) in THF (2 ml) was added 2.5 M *n*-BuLi (0.404 ml, 1.01 mmol) at 0 °C. After stirring for 10 min at 0 °C, aldehyde produced above (0.104 g, 0.51 mmol) in THF (1 ml) was added dropwise at 0 °C. After stirring for 30 min at 0 °C, water (25 ml) was added. The solution was then extracted with ether (50 ml) and dried over MgSO4. The residue was purified by sgc (hexane:ethyl acetate = 10:1) to give (*E*) isomer **9** (112 mg, 73%) and (*Z*) isomer **10** (15 mg, 10%).

(*E*) isomer (**9**): IR $\nu_{\max}$ (neat) cm^{-1}: 3289,2958, 2359, 2225, 2108, 1669, 1628, 844; ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.78 (1H, *dt*, $J=15.3$, 6.9 Hz), 5.78 (1H, *d*, $J=15.3$ Hz), 5.67 (1H, br), 3.13 (2H, *t*, $J=6.3$ Hz), 2.25–2.29 (2H, *m*), 2.14–2.20 (2H, *m*), 1.74–1.83 (1H, *m*), 1.52–1.56 (4H, *m*), 0.91 (6H, *d*, $J=6.9$ Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.1,143.9, 124.3, 88.6, 83.5, 79.8, 66.0, 47.1, 31.5, 28.8, 27.7, 27.5, 20.4, 19.2, −0.2; HREIMS [M]$^+$ m/z: 303.2023 (Calc. 303.2018) for C$_{18}$H$_{29}$NOSi.

(*Z*) isomer (**10**): ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.95 (1H, *dt*, $J=11.4$, 7.5 Hz), 5.69 (1H, *d*, $J=11.4$ Hz), 5.50 (1H, *br*), 3.11 (2H, *t*, $J=6.3$ Hz), 2.63–2.70 (2H, *m*), 2.27–2.31 (2H, *m*), 1.74–1.83 (1H, *m*), 1.49–1.64 (4H, *m*), 0.92 (6H, *d*, $J=6.6$ Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.6, 145.0, 122.9, 88.7, 83.3, 80.1, 65.8, 46.8, 28.8, 28.6, 28.2, 27.9, 20.4, 19.2, −0.1.

4.6.3. (*E*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**3**) and (*Z*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**5**)

To a solution of amide **9** (0.029 g, 0.096 mmol) in THF (1 ml) was added 1 M TBAF (0.144 ml, 0.144 mmol) at 0 °C. After stirring for 30 min, the solvent was removed in vacuo. The residue was purified by sgc (hexane:ethyl acetate = 10:1) to give **3** (0.021g, 95%).

(*E*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**3**): ^{1}H NMR (300 MHz, CDCl$_3$): δ 6.79 (1H, *dt*, $J=15.3$, 6.6 Hz), 5.79 (1H, *d*, $J=15.3$ Hz), 5.62 (1H, *br*), 3.13 (2H, *t*, $J=6.6$ Hz), 2.23–2.29 (2H, *m*), 2.16–2.22 (2H, *m*), 1.97 (1H, *t*, $J=0.9$ Hz) 1.74–1.83 (1H, *m*), 1.52–1.59 (4H, *m*), 0.91 (6H, *d*, $J=6.9$ Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 166.1, 143.8, 124.3, 78.1, 68.6, 65.2, 64.9, 47.1, 31.5, 28.8, 27.6, 27.5, 20.3, 19.0; EIMS 70 eV, m/z (rel. int.): 231 [M]$^+$ (6), 216 [M − CH$_3$]$^+$ (8), 202 (27), 188 [M − C$_3$H$_7$]$^+$ (22), 174 [M − C$_4$H$_9$]$^+$ (16), 160 (21), 131 [M − C$_5$H$_{10}$NO]$^+$ (43), 116 (56), 103 (40), 91 [C$_7$H$_7$]$^+$ (100), 55 (26), 41 (25); HREIMS [M]$^+$ m/z: 231.16260 (Calc. 231.16231) for C$_{15}$H$_{21}$ NO.

(*Z*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**5**): ^{1}H NMR (300 MHz, CDCl$_3$): δ 5.96 (1H, *dt*, $J=11.4$, 7.5 Hz), 5.69 (1H, *d*, $J=11.4$ Hz), 5.49 (1H, *br*), Hz, 1.72–1.86 (1H, *m*), 1.50–1.63 (4H, *m*), 0.92 (6H, *d*, $J=6.6$ Hz); ^{13}C NMR (75 MHz, CDCl$_3$): δ 3.11 (2H, *t*, $J=6.9$ Hz), 2.64–2.72 (2H, *m*), 2.57–2.30 (2H, *m*), 1.95 (1H, *t*, $J=1.2$ 166.6, 145.0, 122.9, 78.5, 68.7, 65.0, 64.7, 46.8, 28.8, 28.5, 28.2, 27.8, 20.4, 19.1); EIMS 70 eV, m/z (rel. int.): 231 [M]$^+$ (4), 216 [M − CH$_3$]$^+$ (5), 202 (14), 188 $[M-C_3H_7]^+$ (11), 174 $[M-C_4H_9]^+$ (12), 159 (18), 131 $[M-C_5H_{10}NO]^+$ (98), 117 (100), 91 $[C_7H_7]^+$ (82), 57 (57), 41 (48).

4.6.4. (E)-N-isobutyl dodeca-2-ene-8,10-diynamide (4)

To a solution of acetal **8** (0.09 g, 0.36 mmol) in THF (5 ml) was added TBAF (1 M solution, 0.542 ml) at 0 °C. The mixture was warmed to rt and stirred for 30 min. Solvent was removed in vacuo. The residue was purified by sgc (hexane:ethyl acetate = 2:1) to give a terminal acetylene that was taken immediately to the next step (0.062 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.35 (1H, *t*, *J* = 5.4 Hz), 3.31 (6H, *s*), 2.27 (2H, *t*, J = 6.6 Hz), 1.96 (1H, *t*, J = 1.2 Hz), 1.53–1.65 (4H, *m*), 1.41–1.49 (2H, *m*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 104.5, 78.3, 68.6, 65.1, 64.8, 52.9, 31.8, 28.0, 24.1, 19.2.

To a solution of the terminal acetylene produced above (0.053 g, 0.29 mmol) in THF (3 ml) was added *n*-BuLi (2.5 M solution, 0.119 ml) at −78 °C. After 10 min, MeI (0.063 ml, 1.02 mmol) was added to the mixture at −78 °C. After adding, the mixture was warmed to rt then HMPA (1.5 ml) was added. After stirring 12 h at rt, ice water (10 ml) was added and the mixture was then extracted with ether (20 ml×3). The organic layer was washed with water and dried ($MgSO_4$). The residue was purified by sgc (hexane:ethyl acetate = 3:1) to give the methylated acetylene that was taken immediately on to the next step (0.045 g, 80%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.30 (1H, *t*, *J* = 5.7 Hz), 3.26 (3H, *s*), 2.20 (2H, *t*, *J* = 6.9 Hz), 1.84 (3H, *s*), 1.49–1.59 (4H, *m*), 1.33–1.44 (2H, *m*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 104.9, 77.6, 72.8, 65.5, 64.0, 51.2, 33.1, 28.6, 23.4, 18.7, 4.2; HREIMS $[M]^+$ *m/z*: 194.1314 (Calc. 194.1307) for $C_{12}H_{18}O_2$.

To a solution of the methylated acetylene produced above (0.045 g, 0.23 mmol) in Me_2CO/H_2O (5 ml/0.5 ml) was added pTSA (0.01 g, 0.05 mmol) at rt. After stirring for 12 h at rt, the solvent was removed. Water (30 ml) was added and the mixture extracted with ether (50 ml), washed with sat $NaHCO_3$, brine, and dried ($MgSO_4$). The residue was purified by sgc (hexane:ethyl acetate = 4:1) to give an aldehyde that was taken on to the next step (0.030 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.75 (1H, *t*, *J* = 1.5 Hz), 2.45 (1H, *td*, *J* = 7.2, 1.5 Hz), 2.27 (2H, *t*, J = 6.9 Hz), 1.84 (3H, *s*), 1.49–1.59 (4H, *m*), 1.33–1.44 (2H, *m*); ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.1, 77.8, 73.4, 65.3, 63.9, 44.7, 26.3, 21.4, 19.1, 4.3.

To a solution of triphenyl-(*N*-isobutylcarboxamidomethyl)-phosphonium bromide (0.165 g, 0.4 mmol) in THF (2 ml) was added 2.5 M *n*-BuLi (2.5 M, 0.16 ml) at 0 °C. After stirring for 10 min at 0 °C, the aldehyde produced above (0.03 g, 0.20 mmol) in THF (1 ml) was added dropwise at 0 °C. After stirring for 30 min at 0 °C, water (25 ml) was added and the mixture was extracted with ether (50 ml), and dried ($MgSO_4$). The residue was purified by sgc (hexane: ethyl acetate = 10:1) to give **4** (0.033 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.68 (1H, *dt*, *J* = 15.3, 6.9 Hz), 5.78 (1H, *d*, *J* = 15.5 Hz), 5.56 (1H, *br s*), 3.14 (2H, *t*, J = 6.3 Hz), 2.15–2.27 (4H, *m*), 1.90 (3H, *s*), 1.73, 1.83 (1H, *m*), 1.53–1.59 (4H, *m*), 0.92 (6H, *d*, J = 6.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.2, 144.1, 124.2, 76.5, 73.5, 65.9, 64.7, 47.1, 31.6, 28.8, 27.9, 27.4, 20.4, 19.2, 4.4; HREIMS $[M]^+$ *m/z*: 245.1784 (Calc. 245.1780) for $C_{16}H_{23}ON$; EIMS 70 eV, *m/z* (rel. int.): 245 $[M]^+$ (13), 230 $[M\text{-}CH3]^+$ (12), 216 (47), 202 (32), 188 $[M-C_4H_9]^+$ (20), 173 (30), 145 $[M-C_5H_{10}NO]^+$ (90), 131 (63), 117 (100), 105 (59), 91 $[C_7H_7]^+$ (61), 77 (55), 57 (35), 41 (38).

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