



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.

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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. angust-*

ifolia (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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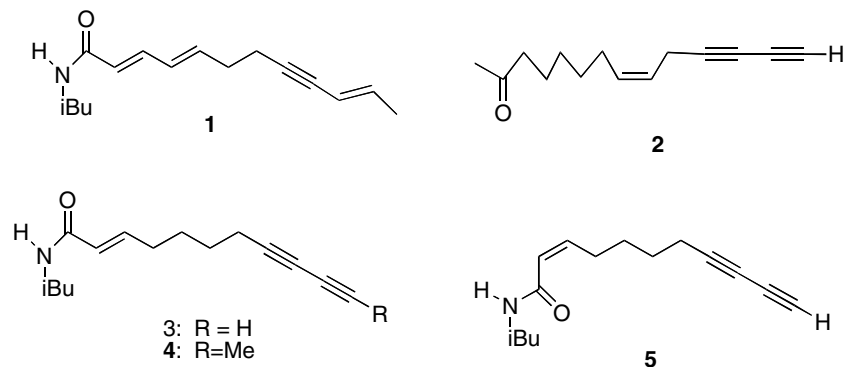


Fig. 1. Typical acetylenes found in *Echinacea* species: (*E,Z,E*)-*N*-isobutyl dodeca-2,4,10-triene-8-ynamide (**1**), (*Z*)-tetradeca-8-ene-11,13-diyne-2-one (**2**), (*E*)-*N*-isobutyl undeca-2-ene-8,10-diyamide (**3**), (*E*)-*N*-isobutyl dodeca-2-ene-8,10-diyamide (**4**), (*Z*)-*N*-isobutyl undeca-2-ene-8,10-diyamide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

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

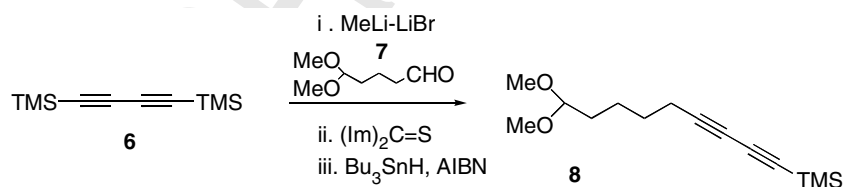
55 2. Results and discussion

56 2.1. Synthesis of diacetylenic isobutylamides

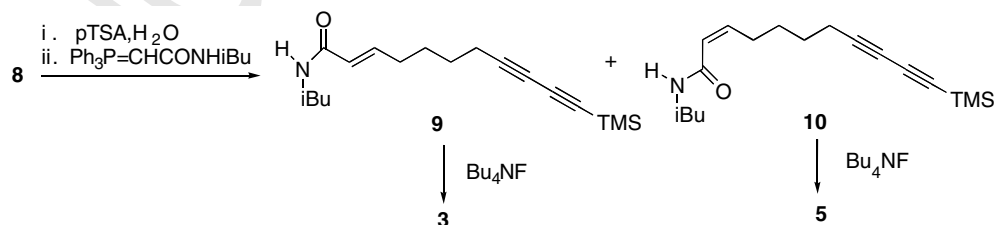
57 Our route began with the construction of acetal **8**
58 (Scheme 1) from commercially available bis-trimethylsilyldiacetylene (**6**) and aldehyde **7** that was readily
59 available from the ozonolysis of cyclopentene by the
60 method of Schreiber (Schreiber et al., 1982). Generation
61 of the monoanion from **6** with methyl lithium–lithium
62 bromide complex in THF at 0 °C followed by reaction
63 at –78 °C with aldehyde **7** afforded a propargylic alcohol in 88% isolated yield (Holmes and Jones, 1980). De-
64 oxygenation was achieved by reaction of the alcohol
65
66

with thiocarbonyldiimidazole (CH₂Cl₂, 25 °C) followed
67 by treatment with two equivalents of tributyltin hydride
68 and AIBN at 80 °C in toluene to produce acetal **8** in 51%
69 yield over two steps (Gunji and Vasella, 2000). The use
70 of larger quantities of tributyltin hydride should be
71 avoided since addition to the acetylene occurred. Al-
72 though alkylation of the anion of the diacetylene would
73 have been more direct, several attempts to effect the di-
74 rect alkylation provided only low yields of the desired
75 product.
76

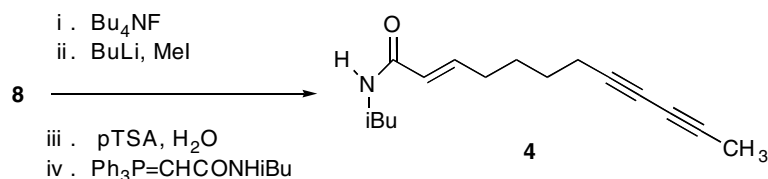
Acetal **8** was the key intermediate for the syntheses of
77 amides **3–5** (Scheme 2). Hydrolysis of the acetal using
78 pTSA in aqueous acetone at ambient temperature gave
79 almost a quantitative yield of aldehyde. The aldehyde
80 reacted with the amide phosphorane to afford *E*-isomer
81 **9** in 73% yield (Barrett et al., 1996). Approximately 10%
82 of the *Z*-isomer **10** was also formed and was readily sep-
83 arable from the *E*-isomer by silica gel flash chromatog-
84



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**.

85 raphy. The reaction of *E*-amide **9** with tetrabutylammo-
 86 nium fluoride (TBAF) in THF at 0 °C produced **3** in
 87 95% yield. The reaction of *Z*-isomer **10** generated com-
 88 pound **5** in 97% yield.

89 The synthesis of **4** from **8** (Scheme 3) began with a de-
 90 silylation reaction using TBAF followed by methylation
 91 of the resulting terminal acetylene with *n*-butyl lithium
 92 and methyl iodide. The latter reaction was very slow
 93 and worked better when an equivalent of hexamethyl-
 94 phosphoric triamide was added after generation of the
 95 acetylide anion. Hydrolysis of the acetal followed by
 96 Wittig reaction afforded diacetylene **4** in 46% yield.
 97 The proton and carbon NMR spectra of the synthesized
 98 amides **3–5** were identical to the spectra reported by
 99 Ramsewak (Ramsewak et al., 1999), and both their
 100 HPLC retention volumes and UV spectra were identical
 101 to those from natural materials (Fig. 2(a)). The three
 102 amides were well separated by the solvent system em-
 103 ployed. Their elution sequence on reversed phase C18
 104 column was influenced by the chain length and the ster-
 105 eochemistry of the double bond at carbon 2, with
 106 amides **3**, **5** and **4** eluted at 17.6 min, 20.2 min and
 107 21.2 min successively. The three amides demonstrated
 108 very similar UV spectra, all with an absorption maxi-
 109 mum at 210 nm caused by the α,β unsaturated amide
 110 chromophore, which agrees with that reported by Bauer
 111 and Remiger (1989).

112 The identity of amides **3–5** was further confirmed by
 113 comparison of their retention times and mass spectra us-
 114 ing GC–MS (Fig. 2(b)). They were well separated by
 115 GC, with amide **5** eluting at 23.1 min followed by amide
 116 **3** and **4** at 25.1 min and 28.5 min, respectively. EIMS
 117 analysis afforded characteristic mass spectrum for each
 118 of the amides, i.e. amides **3**, **4** and **5** gave molecular ions
 119 at m/z 231, 245 and 231, which were calculated for
 120 $\text{C}_{15}\text{H}_{21}\text{ON}$, $\text{C}_{16}\text{H}_{23}\text{ON}$ and $\text{C}_{15}\text{H}_{21}\text{ON}$, respectively.
 121 Molecular ion and many fragment ions of amide **4** were
 122 14 mass units greater than those of amide **3** and **5** due to
 123 the terminal methyl group. The mass spectra of amide **3**
 124 and **5** are very similar but can be distinguished by rela-
 125 tive abundance of some of the fragment ions, eg. frag-
 126 ments $m/z=131$ ($[\text{M}-\text{C}_5\text{H}_{10}\text{NO}]^+$) and $m/z=91$
 127 ($[\text{C}_7\text{H}_7]^+$). Both GC retention times and mass spectra
 128 for the synthetic compounds were identical to those
 129 from the natural materials.

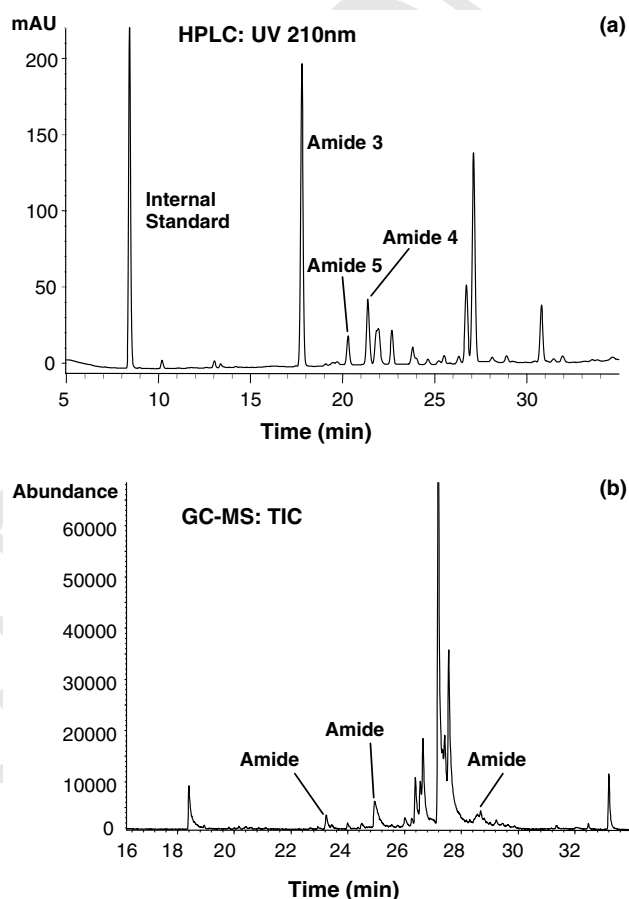


Fig. 2. HPLC chromatogram (a) and GC–MS TIC chromatogram (b) obtained from a 95% ethanolic extract of 3-month-old *E. angustifolia* roots, indicating diacetylenic isobutylamides **3–5**.

2.2. Characterization and distribution of the diacetylenic isobutylamides in *Echinacea* 130 131

Monoene-type isobutylamides **3–5** were first reported 132 in *E. angustifolia* roots (Bauer et al., 1989) and later 133 were identified in roots of *E. tenesseeensis* (Bauer 134 et al., 1990) and *E. simulata* (Bauer and Foster, 1991). 135 They are often used as one of the diagnostic patterns 136 for identification of *E. angustifolia* roots in commercial 137 preparations (Bauer, 1998). Binns et al. (2002) have re- 138 ported that diacetylenic isobutylamides are present to 139 some extent in all *Echinacea* species, however, in that re- 140 port HPLC peaks were identified as diacetylenic isobu- 141

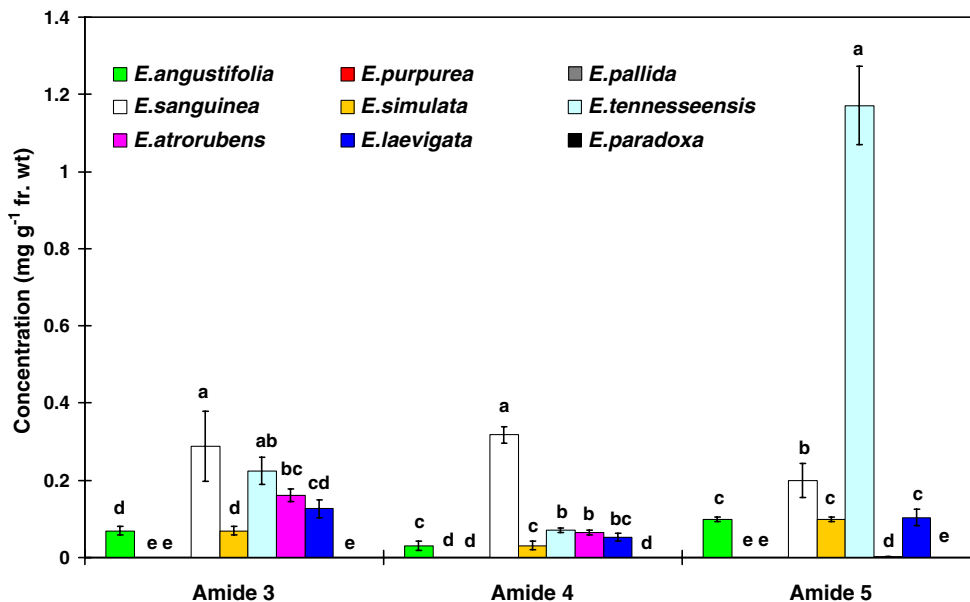


Fig. 3. Concentration of diacetylenic isobutylamides 3–5 in 6-month-old roots of nine species of *Echinacea*. Error bars indicate standard deviations of means of triplicate experiments. For each diacetylenic isobutylamide (3, 4 or 5), different letters indicate a significant difference ($p < 0.05$).

142 tylamides based solely on their UV spectra and their re-
 143 tention times as compared to those of non-diacetylenic
 144 isobutylamide standards.

145 Using the authentic diacetylenic isobutylamides 3–5,
 146 we combined GC–MS and HPLC to identify and quan-
 147 tify their distribution in plants. The mean levels of
 148 amides 3–5 in roots from 6-month-old plants from nine
 149 *Echinacea* species are presented in Fig. 3. Our results
 150 show that of the 9 *Echinacea* species, compounds 3–5
 151 present in six species: *E. angustifolia*, *E. sanguinea*, *E.*
 152 *simulata*, *E. tennesseensis*, *E. atrorubens* and *E. laevigata*.
 153 Among these six species, the highest concentrations of
 154 amide 3 (0.29 ± 0.02 mg g⁻¹ fr. wt) and amide 4
 155 (0.32 ± 0.03 mg g⁻¹ fr. wt) are found in *E. sanguinea*,

156 whereas *E. tennesseensis* contains the highest amount
 157 of amide 5 (1.2 ± 0.1 mg g⁻¹ fr. wt). Studies of the distri-
 158 butions of the three amides in different organs of *E. an-*
 159 *gustifolia* indicate they are present mainly in roots,
 160 have reduced abundance in flowers, and are not detecta-
 161 ble (the limit of HPLC detection for all 3 amides is ap-
 162 proximately $0.02 \mu\text{g ml}^{-1}$) in leaves (Fig. 4). Moreover,
 163 the ratio of amides 3–5 in *E. angustifolia* roots changes
 164 with development. In 3-month-old roots, the ratio of
 165 amide 3 to its isomer amide 5 was 1:9, whereas in 6-
 166 month-old roots this ratio was about 2:3, a difference sig-
 167 nificant at $p < 0.01$. This indicates that isomerisation of
 168 the 2-monoene portion of these amides may be regulated
 169 during root growth and development.

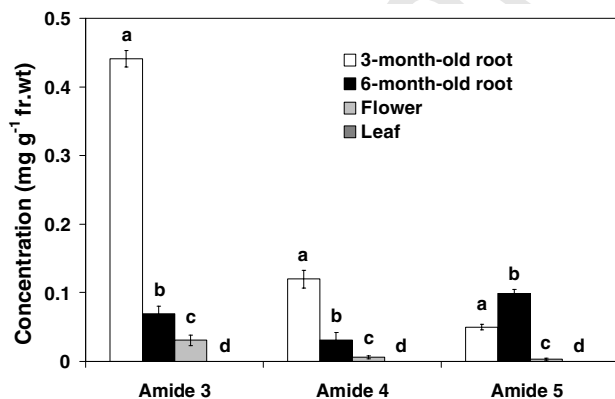


Fig. 4. Concentration of diacetylenic isobutylamides 3–5 in *E. angustifolia* roots from 3 and 6-month-old plants, and flowers, and leaves of 3-month-old plants. Error bars indicate standard deviations of means of triplicate experiments. For each diacetylenic isobutylamide (3, 4 or 5), different letters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

3. Concluding remarks

171 Diacetylenic isobutylamides 3–5 have been synthe-
 172 sized in eight steps from cyclopentene by direct and flex-
 173 ible synthetic routes. The presence of these amides in
 174 *Echinacea* has been confirmed by comparison of reten-
 175 tion times, UV spectra and mass spectra using HPLC
 176 and GC–MS. They are distributed widely in *Echinacea*,
 177 being present in at least six of the nine species (*E. an-*
 178 *gustifolia*, *E. sanguinea*, *E. simulata*, *E. tennesseensis*, *E. at-*
 179 *rorubens* and *E. laevigata*) examined. The abundance
 180 of these compounds varies with organ type and plant
 181 age. Extension of this work to the syntheses of other
 182 members of this class of amides and their natural distri-
 183 bution will further define the metabolic variation among
 184 and within *Echinacea* species.

185 **4. Experimental**186 *4.1. General analytical procedures*

187 Tetrahydrofuran and Et₂O were distilled from sodi-
 188 um benzophenone ketyl, whereas CH₂Cl₂, C₆H₆ and di-
 189 isopropylamide were distilled over calcium hydride. All
 190 experiments were performed under argon atmosphere
 191 unless otherwise noted. Organic extracts were dried over
 192 anhydrous MgSO₄. Infrared spectra were obtained on a
 193 Perkin–Elmer model 1320 spectrophotometer. Nuclear
 194 magnetic resonance experiments were performed with
 195 either a Varian 300 MHz or Bruker 400 MHz instru-
 196 ment. High resolution mass spectra were recorded on
 197 a Kratos model MS-50 spectrometer and low resolution
 198 mass spectra were performed with a Finnegan 4023 mass
 199 spectrometer. Sgc is silica gel flash column chromatogra-
 200 phy.

201 *4.2. Plant material and extraction*

202 Seedlings of *E. angustifolia* (Accession 631267), *E.*
 203 *purpurea* (Accession 631307), *E. pallida* (Accession
 204 631293), *E. sanguinea* (Accession A23878), *E. simulata*
 205 (Accession 631249), *E. tennesseensis* (Accession
 206 631325), *E. atrorubens* (Accession 631262), *E. laevigata*
 207 (Accession 631312) and *E. paradoxa* (Accession
 208 631301) were provided by Dr. Mark P. Widrechner at
 209 the USDA-ARS North Central Regional Plant Intro-
 210 duction Station. Plants were grown under ambient light
 211 in a greenhouse in 7.5 inch pots in soil (50% Canadian
 212 Peat Moss+40% Perlite+10% mineral soil), at 22–25
 213 °C, with daily watering. Pots were laid out in a com-
 214 pletely randomized design generated from random digits
 215 table (Clarke and Kempson, 1997) on a single green-
 216 house bench. Plant samples were harvested from 3-
 217 month-old and 6-month-old plants between 9 and 10
 218 a.m, to standardize plant material with respect to possi-
 219 ble diurnal variations in isobutylamide content. Specifi-
 220 cally, we used: recently mature leaves expanded to over
 221 90% of their final length and located in the mid section
 222 of stems; flowers with 75–90% of the disc florets open,
 223 fully expanded ligulate florets and intensive pollen grain
 224 release; and entire roots. After harvest, plant material
 225 was immediately ground to a fine powder in liquid N₂
 226 in a mortar and pestle and stored in liquid N₂ until ex-
 227 traction. An aliquot of this powdered plant material (0.3
 228 g fr. wt per sample) was further ground in liquid N₂ in
 229 a mortar and pestle with addition of 25 μl (1 mg ml⁻¹) 7-
 230 hydroxy-(*E*)-*N*-isobutylundeca-2-ene-8,10-diynamide
 231 (C₁₅H₂₁O₂) as an internal standard, then with 1 ml of
 232 95% ethanol for 2 min, and the resultant suspension
 233 was transferred to a capped tube. 95% ethanol (2 ml)
 234 was used to rinse the mortar and this was added to
 235 the suspension. The tube was vortexed 30 sec, and cen-
 236 trifuged 1 min at 12,000g. The supernatant (hence re-

ferred to as ethanol extract) was filtered through a 237
 0.22 μm PTFE filter (Alltech, IL). All experiments were 238
 performed in triplicate on independently extracted plant 239
 samples from three individual plants. 240

241 *4.3. HPLC analysis*

242 Ethanol extract (15 μl) was injected into a Beckman 242
 Coulter HPLC with a 508 autosampler, 126 pump con- 243
 trol and 168 UV-photodiode array detector (PDA) con- 244
 trolled by 32karat™ software (Version 5.0), and a 245
 YMC-Pack ODS-AM RP C18 (250×4.6 mm, 5 μm) col- 246
 umn (Waters, MA). The solvent system used was 247
 CH₃CN/H₂O at a flow rate of 1.0 ml/min following a 248
 linear gradient of 40–80% CH₃CN in H₂O over 45 249
 min. Online UV spectra were collected between 200– 250
 400 nm. Amides were quantified based on the internal 251
 standard because they have the same UV absorption 252
 at 210 nm. The limit of HPLC detection for all three 253
 amides is approximately 0.02 μg ml⁻¹. 254

255 *4.4. GC–MS analysis*

256 GC–MS analyses were performed with a GC series 256
 6890 from Agilent (Palo Alto, CA) coupled with a 257
 5973 Agilent mass detector operating in the EI mode 258
 (70 eV), using a HP- 1 silica capillary column (30 259
 m×0.32 mm i.d.; film thickness 0.25 μm), and helium 260
 as the carrier gas. Extracts were evaporated to dryness 261
 under N₂ and resuspended in chloroform (2 ml). The 262
 chloroform-dissolved sample (1 μl) was injected using 263
 splitless injection mode. The temperature of both the in- 264
 jector and detector was at 250 °C. The column temper- 265
 ature was programmed to increase after 2 min from 80 266
 to 260 °C at a rate of 5 °C/min, then held for 10 min, 267
 up to 320 °C at a rate of 5 °C/min, then held for 5 268
 min. Resulting chromatograms were integrated by Agi- 269
 lent's HP enhanced ChemStation™ G1701 BA version 270
 B.01.00 software. Peaks were identified by their mass 271
 spectra and retention times. 272

273 *4.5. Statistical analysis*

274 Statistical analyses were performed using SAS soft- 274
 ware version 8.02 (SAS Institute Inc., Cary, NC). One- 275
 way analysis of variance followed by the Tukey test 276
 was used to compare means. Significance of difference 277
 was defined at *p*<0.05. 278

279 *4.6. Synthesis of amides*280 *4.6.1. 9,9-Dimethoxy-1-trimethylsilyl-1,3-nonadiyne (8)*

281 To a solution of 1,4-bis(trimethylsilyl)butadiyne (1.63 281
 g, 8.4 mmol) in 10 ml of THF was added 1.5 M 282
 MeLi–LiBr (1.5 M solution, 5.58 ml) at 0 °C. The mix- 283
 ture was warmed up to rt. After stirring for 3 h at rt the 284

285 mixture was cooled to $-78\text{ }^{\circ}\text{C}$. To the mixture was add-
 286 ed aldehyde **7** (0.50 g, 3.4 mmol) in THF. After stirring
 287 for 45 min at $-78\text{ }^{\circ}\text{C}$, water (50 ml) was added. The mix-
 288 ture was then extracted with ether (50 ml), washed with
 289 brine, and dried (MgSO_4). The residue was purified by
 290 sgc (hexane:ethyl acetate=4:1) to give the product
 291 (0.793 g, 88%). ^1H NMR (300 MHz, CDCl_3): δ 4.43
 292 (1H, *q*, $J=5.1$ Hz), 4.37 (1H, *t*, $J=5.7$ Hz), 3.32 (6H,
 293 *s*), 1.87 (1H, *br s*), 1.71–1.78 (2H, *m*), 1.58–1.68 (2H,
 294 *m*), 1.48–1.56 (4H, *m*), 0.19 (9H, *s*); ^{13}C NMR (75
 295 MHz, CDCl_3): δ 104.5, 87.6, 87.5, 78.9, 69.9, 62.6,
 296 52.9, 37.3, 32.2, 20.4, -0.29 .

297 To a solution of the compound produced above (0.71
 298 g, 2.65 mmol) in CH_2Cl_2 (10 ml) was added 1,1-thi-
 299 ocarbonyldiimidazole (0.94 g, 5.3 mmol) at rt. After stir-
 300 ring for 12 h, the solvent was removed in vacuo. The
 301 residue was purified by sgc (hexane:ethyl acetate=3:1)
 302 to give the product (0.86 g, 86%). ^1H NMR (300
 303 MHz, CDCl_3): δ 8.27 (1H, *t*, $J=0.9$ Hz), 7.56 (1H, *t*,
 304 $J=1.5$ Hz), 6.98 (1H, *q*, $J=0.9$ Hz), 5.99 (1H, *t*, $J=6.6$
 305 Hz), 4.33 (1H, *t*, $J=5.1$ Hz), 3.27 (6H, *s*), 1.98–2.01
 306 (2H, *m*), 1.55–1.64 (4H, *m*), 0.15 (9H, *s*).

307 To a solution of the thioimidazolide produced above
 308 (0.91 g, 2.4 mmol) in toluene was added AIBN (0.039 g,
 309 0.24 mmol) and Bu_3SnH (0.71 ml, 2.64 mmol) at rt. The
 310 mixture was boiled at $80\text{ }^{\circ}\text{C}$ for 1 h. It was cooled to rt
 311 and solvent was removed in vacuo. The residue was pu-
 312 rified by sgc (hexane:ethyl acetate=10:1) to give the ac-
 313 etal **8** (0.42 g, 69%). ^1H NMR (400 MHz, CDCl_3): δ 4.34
 314 (1H, *t*, $J=5.6$ Hz), 3.30 (6H, *s*), 2.27 (1H, *t*, $J=6.8$ Hz),
 315 1.46–1.61 (4H, *m*), 1.39–1.45 (2H, *m*), 0.18 (9H, *s*); ^{13}C
 316 NMR (100 MHz, CDCl_3): δ 104.5, 88.6, 83.3, 79.9,
 317 65.9, 52.9, 32.2, 28.1, 24.1, 19.4, -0.12 ; HREIMS $[\text{M}]^+$
 318 m/z : 252.1549 (Calc. 252.1546) for $\text{C}_{14}\text{H}_{24}\text{O}_2\text{Si}$.

319 4.6.2. (*E*)-*N*-isobutyl 11-trimethylsilyl-undeca-2-ene-
 320 8,10-diynamide (**9**) and (*Z*)-*N*-isobutyl 11-trimethylsi-
 321 ly-undeca-2-ene-8,10-diynamide (**10**)

322 To a solution of acetal **8** (0.11 g, 0.44 mmol) in
 323 $\text{Me}_2\text{CO}/\text{H}_2\text{O}$ (5 ml/0.5 ml) was added pTSA (0.009 g,
 324 0.044 mmol) at rt. After stirring for 12 h at rt, the sol-
 325 vent was removed. Water (25 ml) was added and the
 326 mixture was extracted with ether (50 ml), washed with
 327 sat NaHCO_3 , brine, and dried (MgSO_4). The residue
 328 was purified by sgc (hexane:ethyl acetate=4:1) to give
 329 an aldehyde that was taken on to the next step (0.081
 330 g, 89%). IR ν_{max} (neat) cm^{-1} : 2958, 2359, 2225, 2108,
 331 1708, 1250, 846; ^1H NMR (300 MHz, CDCl_3): δ 9.75
 332 (1H, *t*, $J=1.8$ Hz), 2.45 (2H, *td*, $J=6.9, 1.8$ Hz), 2.30
 333 (2H, *t*, $J=6.9$ Hz), 1.68–1.78 (2H, *m*), 1.50–1.60 (2H,
 334 *m*), 0.14 (9H, *s*); ^{13}C NMR (75 MHz, CDCl_3): δ 202.1,
 335 88.5, 83.7, 79.3, 66.2, 43.4, 27.7, 21.4, 19.3, -0.1 ;
 336 HREIMS $[\text{M}]^+$ m/z : 206.1130 (Calc. 206.1127) for
 337 $\text{C}_{12}\text{H}_{18}\text{OSi}$.

338 To a solution of triphenyl-*N*-isobutylcarboxamido-
 339 methyl-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 $0\text{ }^{\circ}\text{C}$. After stirring for 10 min at $0\text{ }^{\circ}\text{C}$, alde-
 hyde produced above (0.104 g, 0.51 mmol) in THF (1
 ml) was added dropwise at $0\text{ }^{\circ}\text{C}$. After stirring for 30
 min at $0\text{ }^{\circ}\text{C}$, water (25 ml) was added. The solution
 was then extracted with ether (50 ml) and dried over
 MgSO_4 . 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 ν_{max} (neat) cm^{-1} : 3289, 2958, 2359,
 2225, 2108, 1669, 1628, 844; ^1H 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 $[\text{M}]^+$ m/z : 303.2023 (Calc. 303.2018)
 for $\text{C}_{18}\text{H}_{29}\text{NO}_2\text{Si}$.

(*Z*) isomer (**10**): ^1H 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 $0\text{ }^{\circ}\text{C}$. 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**): ^1H
 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 $[\text{M}]^+$ (6), 216 $[\text{M}-\text{CH}_3]^+$ (8), 202 (27),
 188 $[\text{M}-\text{C}_3\text{H}_7]^+$ (22), 174 $[\text{M}-\text{C}_4\text{H}_9]^+$ (16), 160 (21),
 131 $[\text{M}-\text{C}_5\text{H}_{10}\text{NO}]^+$ (43), 116 (56), 103 (40), 91
 $[\text{C}_7\text{H}_7]^+$ (100), 55 (26), 41 (25); HREIMS $[\text{M}]^+$ m/z :
 231.16260 (Calc. 231.16231) for $\text{C}_{15}\text{H}_{21}\text{NO}$.

(*Z*)-*N*-isobutyl undeca-2-ene-8,10-diynamide (**5**): ^1H
 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 $[\text{M}]^+$ (4), 216 $[\text{M}-\text{CH}_3]^+$ (5),

395 202 (14), 188 [M–C₃H₇]⁺ (11), 174 [M–C₄H₉]⁺ (12),
396 159 (18), 131 [M–C₅H₁₀NO]⁺ (98), 117 (100), 91
397 [C₇H₇]⁺ (82), 57 (57), 41 (48).

398 4.6.4. (*E*)-*N*-isobutyl dodeca-2-ene-8, 10-diynamide (**4**)

399 To a solution of acetal **8** (0.09 g, 0.36 mmol) in THF
400 (5 ml) was added TBAF (1 M solution, 0.542 ml) at 0
401 °C. The mixture was warmed to rt and stirred for 30
402 min. Solvent was removed in vacuo. The residue was pu-
403 rified by sgc (hexane: ethyl acetate=2:1) to give a termi-
404 nal acetylene that was taken immediately to the next
405 step (0.062 g, 96%). ¹H NMR (300 MHz, CDCl₃): δ
406 4.35 (1H, *t*, *J*=5.4 Hz), 3.31 (6H, *s*), 2.27 (2H, *t*,
407 *J*=6.6 Hz), 1.96 (1H, *t*, *J*=1.2 Hz), 1.53–1.65 (4H, *m*),
408 1.41–1.49 (2H, *m*); ¹³C NMR (75 MHz, CDCl₃): δ
409 104.5, 78.3, 68.6, 65.1, 64.8, 52.9, 31.8, 28.0, 24.1, 19.2.

410 To a solution of the terminal acetylene produced
411 above (0.053 g, 0.29 mmol) in THF (3 ml) was added
412 *n*-BuLi (2.5 M solution, 0.119 ml) at –78 °C. After 10
413 min, MeI (0.063 ml, 1.02 mmol) was added to the mixture
414 at –78 °C. After adding, the mixture was warmed to rt
415 then HMPA (1.5 ml) was added. After stirring 12 h at
416 rt, ice water (10 ml) was added and the mixture was then
417 extracted with ether (20 ml×3). The organic layer was
418 washed with water and dried (MgSO₄). The residue was
419 purified by sgc (hexane:ethyl acetate=3:1) to give the
420 methylated acetylene that was taken immediately on to
421 the next step (0.045 g, 80%). ¹H NMR (300 MHz,
422 CDCl₃): δ 4.30 (1H, *t*, *J*=5.7 Hz), 3.26 (3H, *s*), 2.20
423 (2H, *t*, *J*=6.9 Hz), 1.84 (3H, *s*), 1.49–1.59 (4H, *m*),
424 1.33–1.44 (2H, *m*); ¹³C NMR (75 MHz, CDCl₃): δ
425 104.9, 77.6, 72.8, 65.5, 64.0, 51.2, 33.1, 28.6, 23.4, 18.7,
426 4.2; HREIMS [M]⁺ *m/z*: 194.1314 (Calc. 194.1307) for
427 C₁₂H₁₈O₂.

428 To a solution of the methylated acetylene produced
429 above (0.045 g, 0.23 mmol) in Me₂CO/H₂O (5 ml/0.5
430 ml) was added pTSA (0.01 g, 0.05 mmol) at rt. After
431 stirring for 12 h at rt, the solvent was removed. Water
432 (30 ml) was added and the mixture extracted with ether
433 (50 ml), washed with sat NaHCO₃, brine, and dried
434 (MgSO₄). The residue was purified by sgc (hexane:ethyl
435 acetate=4:1) to give an aldehyde that was taken on to
436 the next step (0.030 g, 87%). ¹H NMR (300 MHz,
437 CDCl₃): δ 9.75 (1H, *t*, *J*=1.5 Hz), 2.45 (1H, *td*, *J*=7.2,
438 1.5 Hz), 2.27 (2H, *t*, *J*=6.9 Hz), 1.84 (3H, *s*), 1.49–
439 1.59 (4H, *m*), 1.33–1.44 (2H, *m*); ¹³C NMR (75 MHz,
440 CDCl₃): δ 203.1, 77.8, 73.4, 65.3, 63.9, 44.7, 26.3, 21.4,
441 19.1, 4.3.

442 To a solution of triphenyl-(*N*-isobutylcarboxamido-
443 methyl)-phosphonium bromide (0.165 g, 0.4 mmol) in
444 THF (2 ml) was added 2.5 M *n*-BuLi (2.5 M, 0.16 ml)
445 at 0 °C. After stirring for 10 min at 0 °C, the aldehyde
446 produced above (0.03 g, 0.20 mmol) in THF (1 ml)
447 was added dropwise at 0 °C. After stirring for 30 min
448 at 0 °C, water (25 ml) was added and the mixture was
449 extracted with ether (50 ml), and dried (MgSO₄). The

residue was purified by sgc (hexane: ethyl acetate=10:1) 450
to give **4** (0.033 g, 68%). ¹H NMR (300 MHz, CDCl₃): δ 451
6.68 (1H, *dt*, *J*=15.3, 6.9 Hz), 5.78 (1H, *d*, *J*=15.5 Hz), 452
5.56 (1H, *br s*), 3.14 (2H, *t*, *J*=6.3 Hz), 2.15–2.27 (4H, 453
m), 1.90 (3H, *s*), 1.73, 1.83 (1H, *m*), 1.53–1.59 (4H, *m*), 454
0.92 (6H, *d*, *J*=6.6 Hz); ¹³C NMR (75 MHz, CDCl₃): 455
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*: 457
245.1784 (Calc. 245.1780) for C₁₆H₂₃ON; EIMS 70 eV, 458
m/z (rel. int.): 245 [M]⁺ (13), 230 [M–CH₃]⁺ (12), 216 459
(47), 202 (32), 188 [M–C₄H₉]⁺ (20), 173 (30), 145 460
[M–C₅H₁₀NO]⁺ (90), 131 (63), 117 (100), 105 (59), 91 461
[C₇H₇]⁺ (61), 77 (55), 57 (35), 41 (38). 462

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