129. Caulerpenyne-Amine Reacting System as a Model for *in vivo* Interactions of Ecotoxicologically Relevant Sesquiterpenoids of the Mediterranean-Adapted Tropical Green Seaweed *Caulerpa taxifolia*[1])

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Caulerpenyne (**1**), the most abundant of the ecotoxicologically relevant sesquiterpenoids of the Mediterranean-adapted tropical green seaweed *Caulerpa taxifolia*, was found to react with Et_3N or pyridine in MeOH by initial deprotection of C(1)HO to give oxytoxin 1 (**2a**), previously isolated from the sacoglossan mollusc *Oxynoe olivacea*. With $BuNH_2$, without any precaution to exclude light, **1** gave the series of racemic **3** and **4**, and achiral (4*E*,6*E*)-**5**, (4*E*,6*Z*)-**5**, (4*Z*,6*E*)-**5**, and (4*Z*,6*Z*)-**5** pyrrole compounds, corresponding to formal C(4) substitution, 4,5-β-elimination, and (*E*/*Z*)-isomerization at the C(4)=C(5) and C(6)=C(7) bonds. Changing to $CDCl_3$ as solvent in the dark, **1** gave cleanly, *via* **2a** as an intermediate, **3** and (4*E*,6*E*)-**5**. The latter proved to be prone to (*E*/*Z*)-photoisomerization. Under standard acetylation conditions, **3** gave (4*E*,6*E*)-**5** *via* acetamide **7** as an intermediate. Particular notice is warranted by selective deprotection of **1** at C(1), mimicking enzyme reactions, and unprecedented formation of pyrrole compounds from freely-rotating, protected 1,4-dialdehyde systems.

1. Introduction. – Spreading of the tropical green seaweed *Caulerpa taxifolia* (VAHL) C. AGARDH (Chlorophyta, Caulerpales, Caulerpaceae) in the western part of the Mediterranean basin is the cause of much concern. From recent studies [1], it has emerged that this alga has well adapted to this area, becoming dominant in the invaded zones and expanding rapidly while threatening the *Posidonia oceanica* (L.) Delile ecosystem [2]. Reduction of the biodiversity in such areas was observed, notably at the bacterial level [1] [3].

Mediterranean populations of *C. taxifolia* were found to produce terpenoids that are toxic to prokaryotic and eukaryotic microorganisms involved in the marine food chain [3], as well as to warm-blooded animals [4]. Moreover, one minor terpenoids, 10,11-epoxycaulerpenyne, was observed to induce a dramatic proliferation of *Nicotiana glauca* callus cultures, which can be taken as a model for marine angiosperms [5]. While any direct risk for human health has not clearly emerged to date, these phenomena rank that of Mediterranean-adapted *C. taxifolia* to a case of biological pollution whose consequences, in synergism with other introduced species [6], are difficult to foresee for both the Mediterranean Sea and, on possible natal homing of this algal strain, also the tropics [7].

There is circumstantial evidence suggesting that the bioactive terpenoids produced by *C. taxifolia* are subjected to recognition phenomena as the whole molecule [3] [5]. On the other hand, their multifunctionality is at the basis of the increase in their protozoicidal

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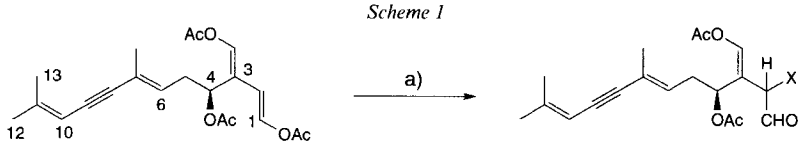
activity following degradation in sea water. This was particularly evident for two of these metabolites, 10,11-epoxycaulerpenyne [3] [7a] and caulerpenynol [7b], but also emerged in caulerpenyne inhibition of the development of fertilized sea urchin eggs [8]. This solicits a clarification of the reactivity of these substances. To this end, we report here on the reactivity of caulerpenyne, the most abundant of the bioactive sesquiterpenoids of *C. taxifolia* [9], towards amines as a model for interaction of this and related metabolites with basic centres of biomolecules *in vivo*.

2. Results and Discussion. – 2.1. *Caulerpenyne and Et_3N or Pyridine.* On adding caulerpenyne to Et_3N in MeOH, oxytoxin 1 (**2a**) was formed (*Scheme 1*). Monitoring this system in CD_3OD by ^{1}H-NMR at room temperature showed that *a*) during the first 30 min from the mixing of the reagents, aside the signals for **1**, only a *d* for C(1)HO for **2b** appeared, and *b*) afterwards, a *s* for another aldehydic proton emerged, presumably indicating formation of the fully deprotected 1,4-dialdehyde. However, after *ca.* 13 h, all aldehydic signals had disappeared completely. In a preparative experiment, **1** and Et_3N in MeOH for 13 h lead to **2a** in *ca.* 40% yield.

Scheme 1



1 **2a** X = H **2b** X = D

a) **1** (0.0027M), Et_3N (0.013M) (or pyridine in large excess) in CD_3OD or MeOH, 22°.

A less basic, non-protic amine such as pyridine, added to **1** in MeOH in the dark, induced similar, though much slower reactions: only after 80 h at room temperature was **2a** clearly detectable (in a 45:100 ratio with respect to caulerpenyne).

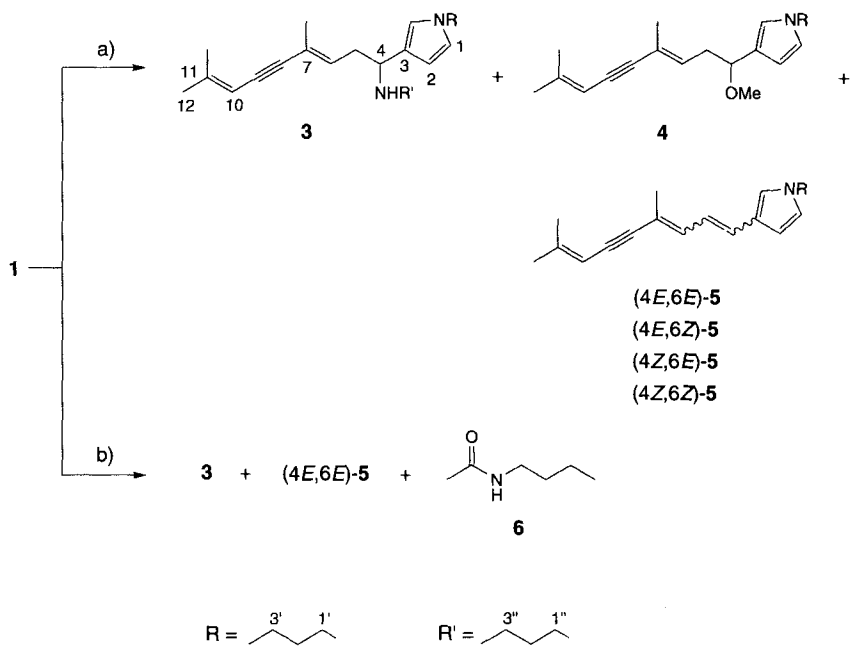
2.2. *Caulerpenyne and $BuNH_2$.* On the treatment with $BuNH_2$ in MeOH (*Scheme 2*), caulerpenyne (**1**) disappeared more rapidly than in the presence of Et_3N. The resulting tarrying mixture was subjected to FC without any precaution to exclude light. Elution with Et_2O afforded a pale-yellow mixture of the less polar pyrrolic compounds **4**, (4*E*,6*E*)-**5**, (4*E*,6Z)-**5**, (4*Z*,6*E*)-**5**, and (4*Z*,6*Z*)-**5**[2]). Changing to AcOEt/MeOH 4:1, the more polar, racemic **3** and tars were eluted.

These products correspond to formal replacement of the AcO group at C(4) by either a BuNH group (→**3**) or a MeO group (→**4**), or to elimination of AcOH and (*E*/*Z*)-isomerization (→(4*E*,6*E*)-**5**, (4*E*,6Z)-**5**, (4*Z*,6*E*)-**5**, (4*Z*,6*Z*)-**5**).

The structures of these products firmly rest on spectral data. In all cases, the molecular ions emerged clearly in EI-MS, while NMR spectra revealed the absence of Ac signals and the replacement of 1,4-diacetoxybutadiene signals by those of a 3-alkylpyrrole ring, which found correspondence in *Ehrlich* reactivity (*cf. Exper. Part* and the *Table*). Substitution at C(4) in both **3** and **4** was deduced from typical ^{1}H- and

[2]) Caulerpenyne (**1**) numbering is used throughout, except for retrieval purposes (*Exper. Part*, where *IUPAC* numbering is used for names).

Scheme 2



a) **1** (0.0027M), $BuNH_2$ (0.018M) in MeOH at 20° in daylight. b) **1** (0.054M), $BuNH_2$ (0.20M) in $CDCl_3$ at NMR-probe temperature (22°) in the dark.

^{13}C-NMR deshielding of H–C(4), as well as from MS fragmentation at C(4)–C(5) (*Exper. Part*). The configurations at the C(4)=C(5) and C(6)=C(7) bonds in the **5**-type products are supported by NOE enhancements data and δ(C) values for CH_3–C(7)[3] (*cf.* *Exper. Part* and the *Table*).

A series of experiments of direct monitoring of the interaction of caulerpenyne (**1**) with $BuNH_2$ was carried out into the ^{1}H-NMR probe. Thus, on mixing **1** and $BuNH_2$ in CD_3OD at 22°, only extremely weak ^{1}H-NMR signals in the aldehyde region could be detected; however, on carrying out the reaction at 15°, aldehydic signals for **2b** and other unidentified aldehydes emerged. This suggests that oxytoxin 1 (**2a**) is an intermediate in the formation of the pyrrolic compounds, although at the higher-temperature conversion to these products is so fast that **2b** does not accumulate[4]).

Compound (4*E*,6*E*)-**5**, while thermally stable in C_6D_6 during 14 h at 70°, proved to undergo clean photoisomerization under light from a Hg lamp through thin *Pyrex* in

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[3]) $MeNH_2$ and caulerpenyne (**1**) in MeCN proved to react similarly, though no attempts were undertaken at product isolation.

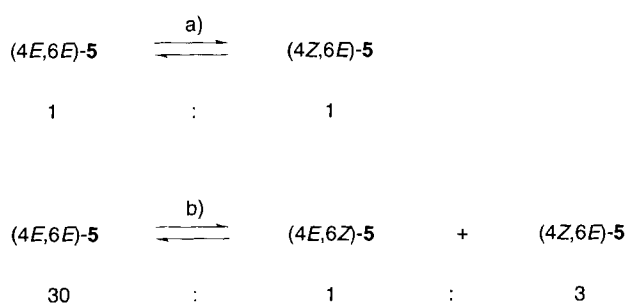
[4]) In support, on mixing **1** with $BuNH_2$ in $CDCl_3$, at short reaction times signals emerged for *N*-butylacetamide (**6**) besides the aldehyde signals for both oxytoxin 1 (**2a**) and another, unidentified aldehydic product, followed, after *ca.* 2 h from the mixing, by the signals for **3** and (4*E*,6*E*)-**5** (*Scheme 2*). In further support, the signals for **2a** (0.02M), mixed with $BuNH_2$ in a three-fold molar excess in $CDCl_3$, disappeared in a few min to give rise to the pyrrolic signals for both **3** and (4*E*,6*E*)-**5**.

Table. *^{13}C-NMR Data for Products* **3**, **4**[a]), *(4*E*,6*E*)-***5***, (4*Z*,6*E*)-***5***, and* **7**

C-Atom	**3**[b])	**4**[c])	(4*E*,6*E*)-**5**[c])	(4*Z*,6*E*)-**5**[c])	**7**[b])[d])	
					major	minor
C(1)	121.30 (*d*)[e])	120.74 (*d*)	122.05 (*d*)	121.90 (*d*)	120.48 (*d*)	120.95 (*d*)
C(2)	108.32 (*d*)	106.46 (*d*)	105.43 (*d*)	105.59 (*d*)[f])	108.44 (*d*)	107.83 (*d*)
C(3)	119.75 (*s*)	119.23 (*s*)	115.51 (*s*)	116.22 (*s*)	123.14 (*s*)	123.48 (*s*)
C(4)	56.95 (*d*)	77.19 (*d*)	127.25 (*d*)	126.26 (*d*)	56.04 (*d*)	51.18 (*d*)
C(5)	35.00 (*t*)	36.33 (*t*)	120.64 (*d*)	123.38 (*d*)	32.97 (*t*)	32.77 (*t*)
C(6)	132.29 (*d*)	133.50 (*d*)	135.86 (*d*)	136.06 (*d*)	134.16 (*d*)	133.17 (*d*)
C(7)	121.30 (*s*)[e])	123.70 (*s*)	122.94 (*s*)	123.02 (*s*)	120.18 (*s*)	120.70 (*s*)
C(8)	95.32 (*s*)	94.82 (*s*)	96.34 (*s*)	93.28 (*s*)	95.52 (*s*)	94.95 (*s*)
C(9)	85.48 (*s*)	84.24 (*s*)	87.17 (*s*)	[g])	85.31 (*s*)	85.85 (*s*)
C(10)	106.53 (*d*)	105.46 (*d*)	105.67 (*d*)	105.87 (*d*)[f])	106.60 (*d*)	106.35 (*d*)
C(11)	146.76 (*s*)	147.39 (*s*)	147.36 (*s*)	147.65 (*s*)	146.70 (*s*)	147.43 (*s*)
C(12)	24.48 (*q*)	24.78 (*q*)	24.85 (*q*)	24.94 (*q*)	24.52 (*q*)	24.52 (*q*)
CH=C(3)	120.43 (*d*)	118.99 (*d*)	120.53 (*d*)	120.32 (*d*)	119.91 (*d*)	118.45 (*d*)
Me–C(7)	18.18 (*q*)	17.70 (*q*)	17.73 (*q*)	23.32 (*q*)	18.08 (*q*)	18.02 (*q*)
Me–C(11)	20.86 (*q*)	20.92 (*q*)	20.98 (*q*)	21.14 (*q*)	20.93 (*q*)	20.93 (*q*)
*Me*O–C(4)		55.86 (*q*)				
C(1′)	49.16 (*t*)	49.35 (*t*)	49.45 (*t*)	49.44 (*t*)	49.11 (*t*)	49.19 (*t*)
C(2′)	33.43 (*t*)	33.51 (*t*)	33.38 (*t*)	33.40 (*t*)	33.65 (*t*)	33.65 (*t*)
C(3′)	19.86 (*t*)	19.90 (*t*)	19.85 (*t*)	19.87 (*t*)	19.93 (*t*)	19.93 (*t*)
C(4′)	13.61 (*q*)	13.64 (*q*)	13.60 (*q*)	13.63 (*q*)	13.65 (*q*)	13.65 (*q*)
C(1″)	45.78 (*t*)				44.93 (*t*)	42.95 (*t*)
C(2″)	29.41 (*t*)				32.53 (*t*)	31.51 (*t*)
C(3″)	20.45 (*t*)				20.59 (*t*)	21.08 (*t*)
C(4″)	13.79 (*q*)				13.81 (*q*)	14.04 (*q*)

[a]) Caulerpenyne numbering [9].
[b]) In C_6D_6.
[c]) In $CDCl_3$.
[d]) Two *s* at 169.47 and 169.32 ppm, and two *q* at 22.08 and 22.30 ppm for two AcN.
[e]) At 121.34 and 120.86 ppm for C(1) and C(7), respectively, in $CDCl_3$.
[f]) Interchangeable data within the same column.
[g]) Not detected.

Scheme 3

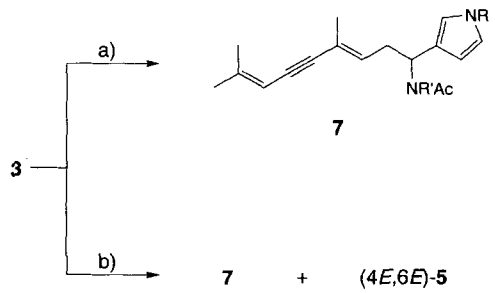


a) With 350-nm light through a *Pyrex* NMR tube during 20 min. b) With a Ne-discharge lamp through a *Pyrex* NMR tube during 6 h.

$CDCl_3$ at room temperature to give a 1:1 mixture with (4*Z*,6*E*)-**5** (*Scheme 3*); on prolonged UV irradiation extensive decomposition occurred. On replacing the Hg lamp with a Ne-discharge lamp, a 3:1:30 mixture (4*Z*,6*E*)-**5**/(4*E*,6*Z*)-**5**/(4*E*,6*E*)-**5** was formed.

Compound **3**, under standard acetylation conditions (Ac_2O/pyridine) in the dark gave acetamide **7**[5]) at low temperature, while on carrying out the acetylation at room temperature both **7** and (4*E*,6*E*)-**5** were formed (*Scheme 4*). This β-elimination of AcNR′ testifies of the great tendency to conjugation in this system.

Scheme 4



a) Ac_2O, Py; 0°. b) Ac_2O, Py; 22°.

3. Conclusions. – Recently, the reactions of primary amines with bioactive compounds carrying 1,4-dialdehyde groups have received considerable attention [10] [11]. Dialdehydes that taste hot, such as polygodial and scalaradial, have the two aldehydic groups rigidly oriented for optimal interaction with a primary amine, thus giving rise to pyrrolic compounds [10]. This chemical reactivity parallels the biological activity, such as feeding repellency, which suggests that these properties are related to covalent interactions of the 1,4-dialdehydic compounds with functional biomolecules. Also γ-hydroxybutenolactones were found to react with a primary amine to give rise to γ-(alkylamino)butenolactones, which explains inactivation of phospholipase A_2 by these dialdehydes, and thus their anti-inflammatory properties [11].

We have shown here that formation of pyrrolic compounds from a 1,4-dialdehyde and a primary amine does not necessarily require that the 1,4-dialdehyde groups be rigidly fixed to a cyclic framework. A linear 1,4-dialdehyde, protected as enol ester such as caulerpenyne (**1**), can form pyrrolic compounds with a primary amine in the flask in fair yields. It may be concluded that it is only when the 1,4-dialdehyde groups are rigidly constrained to be too far apart for mutual interaction with a NH_2 group [10] that pyrrolic compounds are not formed. This suggests the reaction system depicted in *Scheme 2* as a valid model for covalent interactions of caulerpenyne (**1**) and related molecules with living matter [3] [4] [7] [12].

[5]) The existence of **7** in two conformations in 29:21 population ratio for slow rotation of the amide bond, is documented in the *Exper. Part.* s-*cis*-Relation between Me of Ac and C(4) in the minor rotamer rests on a larger Me→H–C(4) NOE effect and a higher field δ(H) resonance of H–C(4) with respect to the major rotamer.

The specificity in C(1)-deprotection of caulerpenyne (**1**) by Et_3N, pyridine, or $BuNH_2$ (*Schemes 1* and *2*) is remarkable and calls for renewed attention on the origin of oxytoxin 1 (**2a**) in extracts of *O. olivacea*. This sacoglossan mollusc feeds on *Caulerpa prolifera*, which contains **1** like most conspecific algae [13]. If, as implied [13], **2a** has enzymatic origin in this mollusc from dietary caulerpenyne, it must be concluded that this was an easy task for the enzyme.

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Experimental Part

1. *General. RP*-HPLC: *Merck Lichrosorb RP-18*, 25 × 1 cm column (7 μm); *CN*-HPLC: *Merck Lichrosorb CN*, 25 × 1 cm column (7 μm). NMR: δ in ppm rel. to internal Me_4Si (= 0 ppm), J in Hz; *Varian XL-300* spectrometer (^1H at 299.94 MHz; ^{13}C at 75.43 MHz), multiplicities from DEPT experiments [14]; $^1H,^1H$ [15] and $^1H,^{13}C$ [16]. HMBC (*via* the heteronuclear multiple-quantum coherence pulse sequence [17a], using a dedicated probe [17b]) are reported as $^1H \rightarrow$ correlated ^{13}C. NOE (= differential NOE) were obtained with 6-s of pre-irradiation and are reported as irradiated proton → % enhancement on observed proton(s). NMR Spectra were taken at 20° unless otherwise stated. MS: EI-MS (*m/z* (%)): *Kratos MS80* mass spectrometer with home-built data system. Photochemical reactions; *Applied Photophysic* semimicro reactor. Evaporations were carried out at reduced pressure and yields are given on reacted substrate.

2. *Collections and Workup. C. taxifolia* was collected in the Baia di Calenzana near Marina di Campo in Elba island at depth 0.5–1 m on 27 October 1994 and was immediately soaked in EtOH. *Caulerpenyne* (**1**) was obtained following a procedure described in [9].

3. *Treatment of* **1** *with Et_3N or Pyridine in MeOH.* To a soln. of **1** (0.025 g, 0.067 mmol) in MeOH (25 ml) was added Et_3N (0.323 mmol), and the mixture was stirred in the dark for either 4 h or 13 h at 22°; workup gave residues whose ^1H-NMR spectra showed, in the first case, **1** and **2a** in 1:1 ratio, while, in the second case, **1** had disappeared completely, and **2a** was isolated by TLC with hexane/Et_2O 1:2 in 40% yield. Treatment of **1** with pyridine in MeOH for 80 h gave **1** and **2a** in 100:45 ratio (^1H-NMR).

4. *Treatment of* **1** *with $BuNH_2$ in MeOH.* To a soln. of **1** (0.050 g, 0.0134 mmol) in MeOH (50 ml), $BuNH_2$ (0.913 mmol) was added, and the mixture was stirred at r.t. for 40 min. The solvent was evaporated and the residue subjected to FC (Et_2O, then AcOEt/MeOH 4:1), collecting a single fraction in each case. The more polar fraction (from AcOEt/MeOH 4:1), subjected to TLC (AcOEt/MeOH/(i-Pr)NH_2 98:10:2), gave **3** (R_f 0.5 band, 13.6%). The less polar fraction (from Et_2O) was subjected to *RP*-HPLC (MeOH/H_2O 9:1) to give **4** (t_R 7 min, 11.4%), (4*E*,6*E*)-**5** (t_R 11 min, 14.8%), and a 10:4:1 mixture (4*E*,6*Z*)-**5**/(4*Z*,6*E*)-**5**/(4*Z*,6*Z*)-**5** (t_R 10 min, 7.8%). This mixture, subjected to HPLC (hexane/AcOEt 99:1), gave (4*E*,6*Z*)-**5**, and a mixture (4*Z*,6*E*)-**5**/(4*Z*,6*Z*)-**5**. Products **3**, **4**, and of **5**-type were observed to develop pink-red, violet, and grey colors, resp., in the *Ehrlich* test.

Data of N-*Butyl[(3*E*)-4,8-dimethyl-1-(1-butylpyrrol-3-yl)nona-3,7-dien-5-yn-1-yl]amine* (**3**): $[\alpha]_D^{20} = 0.0$ ($c = 0.25$, EtOH). UV (EtOH): 270 (14200), 283 (10900). ^1H-NMR (C_6D_6): 6.30 (*dd*, $J(1,2) = 2.4$, $J(1,CH{=}C(3)) = 1.8$, H–C(1)); 6.45 (*dd*, $J(2,1) = 2.4$, $J(2,CH{=}C(3)) = 1.8$, H–C(2)); 3.91 (*dd*, $J(4,5) = 10.2, 4.2$, H–C(4)); 3.38, 3.13 (*m*, 2 H–C(5)); 6.04 (br. *t*, $J(6,5) = 7.5$, H–C(6)); 5.40 (*qq*, $J(10,12) = J(10, Me{-}C(11)) = 1.2$, H–C(10)); 1.46 (br. *d*, $J(12,10) = 1.2$, 3 H–C(12)); 6.67 (*dd*, $J(CH{=}C(3),1) = J(CH{=}C(3), 2) = 1.8$, $CH{=}C(3)$); 2.02 (br. *d*, $J(Me{-}C(7),6) = 1.5$, Me–C(7)); 1.78 (br. *d*, $J(Me{-}C(11),10) = 1.2$, Me–C(11)); 3.19 (*t*, $J(1',2') = 7.2$, 2 H–C(1')); 1.27 (*tt*, $J(2',1') = J(2',3') = 7.2$, 2 H–C(2')); 0.94 (*tq*, $J(3',2') = J(3',4') = 7.2$, 2 H–C(3')); 0.69 (*t*, $J(4',3') = 7.2$, 3 H–C(4')); 2.76, 2.61 (ABX_2, $J(AB) = 10.5$, $J(AX) = J(BX) = 7.5$, 2 H–C(1")); 1.75 (*m*, X_2, 2 H–C(2")); 1.14 (*tq*, $J(3'',2'') = J(3'',4'') = 7.8$), 2 H–C(3")); 0.77 (*t*, $J = 7.8$, 3 H–C(4")). NOE: H–C(1)→9% on H–C(2); H–C(2)→4% on both H–C(1) and H–C(4); 3 H–C(12)→10% on H–C(10). MS: 340 (0.2, M^+), 267 (4, $[M-BuNH_2]^+$), 252 (3), 239 (3), 207 (100, [Bu–NH=CH–N–Bu-pyrrole]$^+$), 124 (8).

*Data of (3*E*)-1-(1-Butylpyrrol-3-yl)-1-methoxy-4,8-dimethylnona-3,7-dien-5-yne* (**4**): $[\alpha]_D^{20} = 0.0$ ($c = 0.1$, EtOH). UV (EtOH): 269 (22000), 283 (17300). ^1H-NMR ($CDCl_3$): 6.58 (*dd*, $J(1,2) = 2.4$, $J(1,CH{=}C(3)) = 1.8$, H–C(1)); 6.07 (*dd*, $J(2,1) = 2.4$, $J(2,CH{=}C(3)) = 1.8$, H–C(2)); 4.10 (*t*, $J(4.5) = 6.7$, H–C(4)); 2.63, 2.51 (*ABXY*, $J(AB) \approx 14$, $J(AX) = J(BX) = 7.3$, $J(AY) = J(BY) = 6.7$, H_A–C(5), H_B–C(5)); 5.84 (*tq*, $J(6,5) = 7.3$,

J(6,*Me*–C(7) = 1.5, H–C(6)); 5.35 (br. *s*, H–C(10)); 1.80 (br. *d*, J(12,10) = 1.2, 3 H–C(12)); 6.57 (*dd*, J(C*H*=C(3),1) = J(C*H*=C(3),2 = 1.8, C*H*=C(3)); 1.79 (br. *d*, J(*Me*–C(7),6) = 1.5, Me–C(7)); 1.88 (br. *s*, Me–C(11)); 3.23 (*s*, MeO); 3.82 (*t*, J(1′,2′) = 7.2, 2 H–C(1′)); 1.73 (*tt*, J(2′,1′) = J(2′,3′) = 7.2, 2 H–C(2′)); 1.30 (*tq*, J(3′,2′) = J(3′,4′) = 7.2, 2 H–C(3′)); 0.92 (*t*, J(4′,3′) = 7.2, 3 H–C(4′)). MS: 299 (0.1, M^+), 267 (2, [M–MeOH]$^+$), 252 (1), 166 (100, [Me–O=CH–(*N*-butylpyrrole)]$^+$), 110 (7).

*Data of (1*E,*3*E*)-1-(1-Butylpyrrol-3-yl)-4,8-dimethylnona-1,3,7-trien-5-yne* ((4*E*,6*E*)-**5**): UV (EtOH): 344 (27100), 258 (8600). ^{1}H-NMR ($CDCl_3$): 6.58 (br. *dd*, J(1,2) = 2.7, J(1,C*H*=C(3)) = 1.8, H–C(1)); 6.30 (*dd*, J(2,1) = 2.7, J(2,C*H*=C(3)) = 1.8, H–C(2)); 6.47 (br. *d*, J(4,5) = 15.0, H–C(4)); 6.64 (*dd*, J(5,4) = 15.0, J(5,6) = 11.0, H–C(5)); 6.46 (br. *d*, J(6,5) = 11.0, H–C(6)); 5.42 (*qq*, J(10,12) = J(10,*Me*–C(11)) = 1.3, H–C(10)); 1.83 (br. *s*, 3 H–C(12)); 6.70 (*dd*, J(C*H*=C(3),1) = J(C*H*=C(3),2) = 1.8, C*H*=C(3)); 1.98 (br. *s*, Me–C(7)); 1.92 (br. *s*, Me–C(11)); 3.81 (*t*, J(1′,2′) = 7.3, 2 H–C(1′)); 1.73 (*tt*, J(2′,1′) = 7.3, J(2′,3′) = 7.5, 2 H–C(2′)); 1.31 (*tq*, J(3′,2′) = J(3′,4′) = 7.5, 2 H–C(3′)); 0.93 (*t*, J(4′,3′) = 7.5, 3 H–C(4′)). NOE: H–C(1)→12% on H–C(2); H–C(2)→10% on H–C(1), 6% on H–C(5); C*H*=C(3)→8% on H–C(4); *Me*–C(7)→9% on H–C(5); 3 H–C(12)→15% on H–C(10). MS: 267 (100, M^+), 252 (56, [M–CH_3]$^+$), 224 (22), 210 (12), 196 (15), 195 (18), 194 (19), 181 (20), 180 (16), 167 (18), 129 (20), 57 (18), 41 (36).

*Data of (4*E,*6*Z)-**5**: UV (EtOH): 341 (27000), 258 (11200). ^{1}H-NMR ($CDCl_3$): 6.57 (br. *dd*, J(1,2) = 2.7, J(1,C*H*=C(3)) = 1.8, H–C(1)); 6.29 (*dd*, J(2,1) = 2.7, J(2,C*H*=C(3)) = 1.8, H–C(2)); 6.46 (br. *d*, J(4,5) = 15.5, H–C(4)); 6.92 (*dd*, J(5,4) = 15.5, J(5,6) = 11.0, H–C(5)); 6.27 (br. *d*, J(6,5) = 11.0, H–C(6)); 5.50 (*qq*, J(10,12) = J(10,*Me*–C(11)) = 1.2, H–C(10)); 1.86 (br. *s*, 3 H–C(12)); 6.68 (*dd*, J(C*H*=C(3),1) = J(C*H*=C(3),2) = 1.8, C*H*=C(3)); 1.95 (br. *s*, Me–C(7)); 2.01 (br. *s*, Me–C(11)); 3.80 (*t*, J(1′,2′) = 7.1, 2 H–C(1′)); 1.72 (*m*, 2 H–C(2′)); 1.29 (*tq*, J(3′,2′) = J(3′,4′) = 7.3, 2 H–C(3′)); 0.92 (*t*, J(4′,3′) = 7.3, 3 H–C(4′)). NOE: *Me*–C(7)→12% on H–C(6); 3 H–C(12)→13% on H–C(10). MS: 267 (100, M^+), 252 (60, [M–CH_3]$^+$), 224 (23), 210 (14), 196 (19), 195 (19), 194 (16), 181 (20), 167 (14), 129 (16), 57 (14), 41 (17).

*Data of the 4:1 Mixture (4*Z,*6*E*)-**5**/(4*Z*,*6*Z)-**5**: UV (EtOH): 343 (27000), 258 (8800). ^{1}H-NMR ($CDCl_3$) of (4*Z*,6*E*)-**5**: 6.60 (br. *dd*, J(1,2) = 2.7, J(1,C*H*=C(3)) = 1.8, H–C(1)); 6.27 (*dd*, J(2,1) = 2.7, J(2,C*H*=C(3)) = 1.8, H–C(2)); 6.26 (br. *d*, J(4,5) = 11.5, H–C(4)); 6.12 (*dd*, J(5,4) = J(5,6) = 11.5, H–C(5)); 7.01 (br. *d*, J(6,5) = 11.5, H–C(6)); 5.43 (br. *s*, H–C(10)); 1.84 (br. *s*, 3 H–C(12)); 6.76 (*dd*, J(C*H*=C(3),1) = J(C*H*=C(3),2) = 1.8, C*H*=C(3)); 1.97 (br. *d*, J(*Me*–C(7),6) = 1.2, Me–C(7)); 1.92 (br. *s*, Me–C(11)); 3.84 (*t*, J(1′,2′) = 7.0, 2 H–C(1′)); 1.74 (*m*, 2 H–C(2′)); 1.31 (*tq*, J(3′,2′) = 7.1, J(3′,4′) = 7.2, 2 H–C(3′)); 0.93 (*t*, J(4′,3′) = 7.2, 3 H–C(4′)). NOE for (4*Z*,6*E*)-**5**: H–C(1)→13% on H–C(2); *Me*–C(7)→17% on H–C(5); 3 H–C(12)→20% on H–C(10). ^{1}H-NMR ($CDCl_3$) of (4*E*,6*Z*)-**5**: 6.58 (br. *dd*, H–C(1)); 6.40 (*dd*, J(5,4) = J(5,6) = 11.5, H–C(5)); 6.86 (br. *d*, J(6,5) = 11.5, H–C(6)); 5.49 (br. *s*, H–C(10)); 1.84 (br. *s*, 3 H–C(12)); 6.72 (*dd*, C*H*=C(3)); 2.00 (br. *s*, Me–C(7)); 1.95 (br. *s*, Me–C(11)); 3.82 (*t*, J = 7.0, 2 H–C(1′)); 0.92 (*t*, J = 7.2, 3 H–C(4′)). MS: 267 (100, M^+), 252 (60, [M–CH_3]$^+$), 224 (24), 210 (15), 196 (23), 195 (22), 194 (17), 181 (23), 167 (15), 129 (19), 57 (19), 41 (23).

5. *Treatment of* **1** *with* $BuNH_2$ *in* CD_3OD. To a soln. of **1** (0.010 g, 0.027 mmol) in CD_3OD (0.5 ml), $BuNH_2$ (0.081 mmol) was added. Recording ^{1}H-NMR spectra every 15 min, complete transformation of **1** was observed in either 45 min at 22° or 120 min at 15°.

6. *Treatment of* **1** *with* $BuNH_2$ *in* $CDCl_3$. To a soln. of **1** (0.010 g, 0.027 mmol) in $CDCl_3$ (0.5 ml), $BuNH_2$ (0.10 mmol) was added and ^{1}H-NMR spectra were recorded at 22° every 30 min. Complete transformation of **1** was observed in 270 min. Then, working in the dark, the soln. was evaporated and subjected to FC as described in *Exper. 4*: (4*E*,6*E*)-**5** and **3** were obtained from Et_2O or AcOEt/MeOH 4:1 fractions, respectively.

7. *Treatment of Oxytoxin 1* (**2a**) *with* $BuNH_2$ *in* $CDCl_3$. To a soln. of **2a** (0.010 mmol) in $CDCl_3$ (0.5 ml), $BuNH_2$ (0.03 mmol) was added while recording ^{1}H-NMR spectra at 20° (the first five spectra every 2 min and then every 15 min). Whereas complete disappearance of the aldehydic signal required 8 min, complete formation of C(4)=C(5)-bearing compounds required 180 min. Workup as in *Exper. 4* gave (4*E*,6*E*)-**5** and **3**.

8. *UV Irradiation of (4*E,*6*E*)-***5*** *in* $CDCl_3$. A soln. prepared from pure (4*E*,6*E*)-**5** (0.004 g) in $CDCl_3$ (0.5 ml) was irradiated in a standard 5-mm *Pyrex* NMR tube by a Ne-discharge lamp. ^{1}H-NMR Spectra taken after 6 h of irradiation revealed the presence of (4*E*,6*E*)-**5**, (4*Z*,6*E*)-**5**, and (4*E*,6*Z*)-**5** in 30:3:1 ratios. In a parallel experiment, pure (4*E*,6*E*)-**5** (0.007 g) in $CDCl_3$ (0.5 ml) was irradiated for 20 min with the 350-nm emission of a Hg lamp through the same NMR tube, by which ^{1}H-NMR spectra revealed the presence of only isomerized (4*Z*,6*E*)-**5** besides residual (4*E*,6*E*)-**4** in 1:1 ratio. Further irradiation caused extensive decomposition.

9. *Treatment of* **3** *with* Ac_2O *in Pyridine*. To a soln. of **3** (0.010 g) in dry pyridine (0.4 ml) in the dark, Ac_2O (0.10 ml) and Et_3N (0.010 ml) were added, and the mixture was stirred at 0° for 4 h. The mixture was then evaporated and subjected to TLC (hexane/Et_2O 1:2) to give **7** (R_f 0.45), which was further purified by HPLC

(hexane/(i-Pr)OH 47:3): **7** (0.009 g, 80%). In a separate run at 22° for 2 h, **7** and (4*E*,6*E*)-**5** were obtained in 4:1 ratio.

Data of N-*Butyl*-N-*[(3*E*)-4,8-dimethyl-1-(1-butylpyrrol-3-yl)nona-3,7-dien-5-yn-1-yl]acetamide* (**7**): $[\alpha]_D^{20} = 0.0$ ($c = 0.25$, EtOH). UV (EtOH): 270 (13000), 283 (10100). Two rotamers were observed in 29:21 ratio by NMR (C_6D_6). For the major rotamer: ^{1}H-NMR: 6.30 (*dd*, $J(1,2) = J(1,CH{=}C(3)) = 2.2$, H–C(1)); 6.20 (submerged, H–C(2)); 6.10 (*t*, $J(4,5) = 8.0$, H–C(4)); 2.82, 2.67 (*m*, 2 H–C(5)); 6.24 (submerged, H–C(6)); 5.42 (br. *s*, H–C(10)); 1.46 (br. *s*, 3 H–C(12)); 6.40 (*dd*, $J(CH{=}C(3),1) = J(CH{=}C(3),2) = 1.8$, $CH{=}C(3)$); 1.99 (br. *s*, Me–C(7)); 1.79 (br. *s*, Me–C(11)); 3.17 (*t*, $J(1',2') = 6.2$, 2 H–C(1')); 1.20 (*m*, 2 H–C(2')); 0.95 (*m*, 2 H–C(3')); 0.71 (*t*, $J(4',3') = 6.9$, 3 H–C(4')); 2.85 (*m*, 2 H–C(1'')); 1.2 (*m*, 2 H–C(2'')); 1.05 (*m*, 2 H–C(3'')); 0.69 (*t*, $J = 7.0$, 3 H–C(4'')); 1.90 (*s*, MeCO). HMBC: H–C(1)→C(2), C(3), *C*–C(3), C(1'); H–C(4)→C(2), C(3), C(5), C(6), *C*–C(3), C(1''), C=O; 3 H–C(12)→C(10), C(11), *C*–C(11); *H*C–C(3)→C(1), C(2), C(3); *Me*–C(7)→C(6), C(7), C(8); *Me*–C(11)→C(10), C(11), C(12); *Me*–C=O→C=O. NOE: *Me*–CO→4% on H–C(4). For the minor rotamer: ^{1}H-NMR 6.30 (*dd*, $J(1,2) = J(1,CH{=}C(3)) = 2.2$, H–C(1)); 6.04 (*dd*, $J(2,1) = J(2,CH{=}C(3)) = 2.2$, H–C(2)); 4.71 (*dd*, $J(4,5) = 8.5, 6.5$, H–C(4)); 2.62, 2.51 (*m*, 2 H–C(5)); 6.08 (submerged, H–C(6)); 5.46 (br. *s*, H–C(10)); 1.47 (br. *s*, 3 H–C(12)); 6.21 (submerged, $CH{=}C(3)$); 1.85 (br. *s*, Me–C(7)); 1.82 (br. *s*, Me–C(11)); 3.19 (*t*, $J(1',2') = 6.0$, 2 H–C(1')); 1.20 (*m*, 2 H–C(2')); 0.95 (*m*, 2 H–C(3')); 0.68 (*t*, $J(4',3') = 7.0$, 3 H–C(4')); 3.3 (*m*, 2 H–C(1'')); 1.6 (*m*, 2 H–C(2'')); 0.95 (*m*, 2 H–C(3'')); 0.81 (*t*, $J = 7.0$, 3 H–C(4'')); 2.08 (*s*, MeCO). HMBC: H–C(1)→C(2), *C*–C(3), C(1'); H–C(4)→C(2), C(3), C(5), *C*–C(3), C(1''), C=O; 3 H–C(12)→C(10), C(11), *C*–C(11); *Me*–C(7)→C(6), C(7), C(8); *Me*–C(11)→C(10), C(11), C(12); *Me*–C=O→C=O. NOE: *Me*–C=O→8%on H–C(4). NMR Spectra in $(CD_3)_2SO$ showed two rotamers in a 31:27 ratio as from integration of *Me*–C=O or H–C(6) at 1.99 (*s*) and 2.09 (*s*) or at 5.59 (br. *t*) and 5.79 (br. *t*) for the major and minor rotamer, respectively. MS: 382 (0.4, M^+), 339 (0.5), 268 (6), 267 (20, $[M - (N\text{-butylacetamide})]^+$), 208 (11), 207 (66), 149 (12), 124 (14), 98 (100).

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