Penicillin Acylase-Mediated Synthesis of 2-Acetyl-1-pyrroline and of 2-Propionyl-1-pyrroline, Key Roast-Smelling Odorants in Food. Inclusion Complexes with β-Cyclodextrin and Their NMR and MS Characterization

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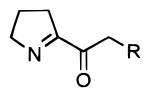
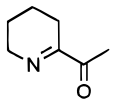
The synthesis of the strong natural roast odorants **1** and **2** is achieved from the C-6 isomeric alcohols **16** and **21** *via* the acetylenic C-6 and C-7 amines **8** and **9.** Key step in the process is the hydrolysis of the *N*-phenylacetamides **12** and **13** by means of immobilized penicillin acylase, which affords the 1-amino-4,5-diketones **14** and **15**, spontaneously ring closing to **1** and **2**. These latter compounds form inclusion complexes with β-cyclodextrin, as demonstrated by NMR measurements in deuterated water and FAB-MS spectra.

2-Acetyl-1-pyrroline (**1**), 2-propionyl-1-pyrroline (**2**), and 2-acetyltetrahydropyridine (**3**) are roast-smelling compounds present in food, with odor threshold at the ppb level.[1] The chemistry of these compounds has received considerable attention since their discovery in foods like cooked rice, pop corn, and bread crust,[2,3,4] in *Pandanus amaryllifolius* Roxb leaves,[5] used in India and in other parts of Asia in the cooking of common rices to impart a resemblance of the aroma of the more costly scented rice and, tentatively, in the urine of tigers, which use **1** for territorial and sexual connotations.[6] Several synthetic approaches to **1** and **2** have been proposed up to now. The existing syntheses of 2-acetyl-pyrroline (**1**) rely on the cyclic intermediates **4**,[7,8] **5** and **6**,[9] or **7**.[10] However, oxidation of **4** to **1** with silver carbonate on Celite in refluxing toluene produces a complex mixture, and the desired product has to be purified by chromatography. Similarly, the obtainment of intermediates **5** and **6**, later reacted with C-1 or C-2 organometallic reagent to produce **1** or **2**, requires the use of *t*-BuOCl and HCN. Finally, the obtainment of **7** involves the use of *t*-BuLi at −40 °C for a long period of time. Reputedly, compound **7** is hydrolyzed to **1** with aqueous hydrochloric acid in unspecified yield.



1 R = H
2 R = CH_3



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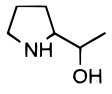

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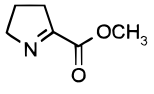

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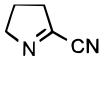

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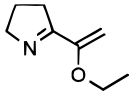

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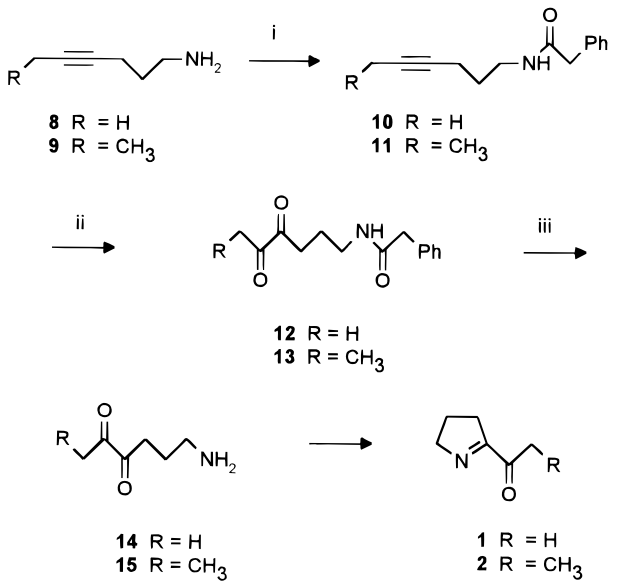
We now report on a new synthesis of **1** and **2** involving the key intermediacy of the N-protected acyclic 1-amino-4,5-diketones **12** and **13**, possessing the carbon skeleton and all the functionalities of **1** and **2** in the correct oxidation state, converted by enzymatic hydrolysis into the amino diketones **14** and **15**, which spontaneously ring close to the desired products.

Scheme 1[a]



[a] Reagents: (i) $PhCH_2COCl$/pyridine/CH_2Cl_2/rt/24 h; (ii) O_3/−78 °C/CH_2Cl_2:MeOH 4:1, and then Me_2S; (iii) immobilized PGA (600 ui/g), pH 7.5/23 °C.

The relevant part of the synthesis of **1** and **2** is outlined in Scheme 1. Starting products are the C-6 and C-7

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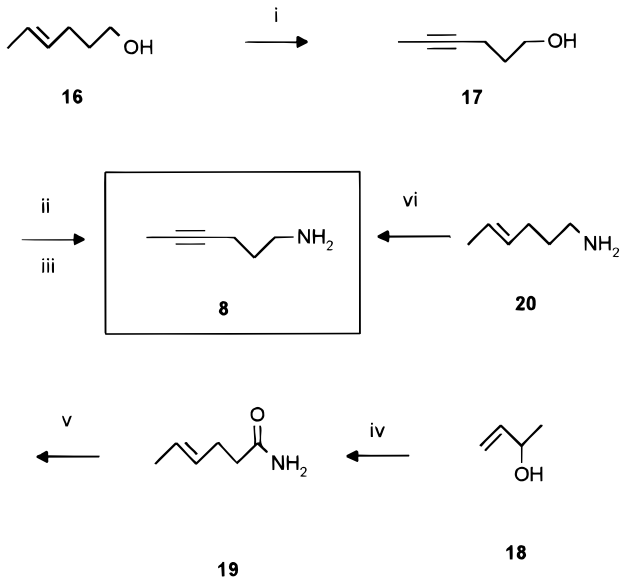
acetylenic amines **8** and **9**, respectively, prepared from easily available starting materials in a number of ways (see below). The oxidation with ozone of the acetylenic moiety of the latter to the 4,5-diketone present in hidden form in **1** and **2** required protection of the primary amine. The protecting group suitable under these circumstances had to be chosen among those removable under mild conditions and not requiring hydrogenolysis, in order to prevent alteration of the sensible α-diketone moiety and/or of the final 2-acyl-1-pyrrolines **1** and **2**. In the light of previous experience,[11] we thought it useful to protect the amino group of **8** and **9** as an amide with phenylacetic acid and to use immobilized penicillin acylase (PGA) as a catalyst in the final hydrolytic deprotection step. In fact, the crystalline C-6 and C-7 *N*-phenylacetyl amides **10** and **11** were oxidatively converted with ozone at low temperature and Me_2S treatment into the 4,5-diketones **12** and **13**, containing all the functionalities present in **1** and **2**. These were revealed when the alcoholic solution of the phenylacetamides **12** and **13** was added dropwise at pH 7.5 to a stirred suspension of PGA immobilized on Eupergit C beads. The hydrolysis of the nitrogen protecting group was quite rapid, and the desired products **1** and **2** were obtained in up to 80% isolated yield and in chemically pure form (GLC/MS and ^{1}H NMR) by bulb-to-bulb vacuum distillation of the residue obtained by careful evaporation of the ether extract of the aqueous phase, from which the beads were separated by decantation. Thus, the important odorants **1** and **2** become accessible in a few steps from the amines **8** and **9**. The relevant part of the synthesis is the enzymatic hydrolysis of the *N*-phenylacetamides **12** and **13**, which proceeds under mild conditions in a very selective way. Indeed, the phenylacetic acid produced in the hydrolytic step remains in the alkaline aqueous phase at the end of the reaction when the final products are recovered by solvent extraction. Moreover, the enzymatic beads can be reused since there is no loss of activity in this nonconventional use of penicillin acylase.

However, it is worth noting that the practical interest of the straightforward synthesis of **1** and **2** from the acetylenic amines **8** and **9** will also depend upon the effectiveness of the preparation of these materials. We tried a number of routes, all purposely based on bromination−dehydrobromination of olefinic materials as a tool for the introduction of the acetylenic moiety. Thus, 1-amino-4-hexyne (**8**), key intermediate in the preparation of roast-smelling **1**, was first obtained from commercially available 4-hexen-1-ol (**16**), a component of banana fruit flavor.[12] This was transformed (Scheme 2), by bromination−dehydrobromination,[13] into the acetylenic carbinol **17**, converted, in turn, into the required amine **8** by treatment with dry ammonia in ethanol of the corresponding 4-nitrobenzenesulfonate in *ca.* 43% yield from **16**. However, 4-hexen-1-ol (**16**) is a rather expensive material. Accordingly, a second approach to **8** was studied (Scheme 2), in which the amide **19** is the relevant intermediate. To this end, 3-buten-2-ol (**18**) was reacted with triethyl orthoformate to provide, under Claisen *ortho*-ester rearrangement, ethyl 4-hexenoate. The ester was hydrolyzed and the resulting acid, *via* the corresponding chloride obtained with $SOCl_2$, afforded upon treatment with aqueous ammonia the desired amide **19**. The latter, upon $LiAlH_4$ reduction, yielded the amine **20**. This material was acetylated and submitted to the bromination−dehydrobromination procedure, to afford directly the required acetylenic amine **8** in *ca.* 8% yield from **18**.

Scheme 2[a]



[a] Reagents: (i) Br_2/CH_2Cl_2, and then KOH/MeOH, reflux; (ii) 4-$NO_2C_6H_4SO_2Cl$/pyridine, rt; (iii) dry NH_3 in EtOH; (iv) $HC(OEt)_3/H^+$/reflux; NaOH in EtOH−H_2O 1:1, reflux, and then H^+; $SOCl_2$, and then addition to 33% aqueous NH_3; (v) $LiAlH_4$/THF, reflux; (vi) Ac_2O/pyridine, Br_2/CH_2Cl_2, and then KOH/MeOH, reflux.

The synthesis of the C-7 acetylenic amine **9** was similarly achieved from a flavor material, *i.e.*, *cis*-3-hexenol (**21**), the so-called leaf alcohol.[14] Chain elongation of the C-6 framework of **21** (Scheme 3) to the C-7 nitrile **22** took place *via* tosylate and KCN displacement. The nitrile moiety of **22** was reduced with AlH_3 to provide the amine **23** converted, as above, into the required amine **9**, in *ca.* 14% overall yield. An alternative approach to **23** from **21** avoiding the use of KCN was also studied. To this end, carbinol **21** was converted into the corresponding bromide upon reaction with Ph_3P/NBS in CH_2Cl_2. The Grignard reagent obtained from the latter in Et_2O was carbonated to the C-7 unsaturated acid **24**. Again, the amide **25** was obtained *via* the acid chloride and treatment with ammonia. Reduction of the latter with $LiAlH_4$ yielded the amine **23** in *ca.* 20% yield from **21**.

Seen together, these results thus show possible synthetic entries to the important flavor materials **1** and **2** from the isomeric flavor materials 4-hexen-1-ol (**16**) and *cis*-3-hexenol (**21**). Most of the reactions involved proceed in high yields but, most important, the last one which gives access to the chemically sensible molecules **1** and **2** occurs under enzymatic catalysis to provide materials possessing very high chemical purity. The present synthesis thus represents another example of the significance for organic synthesis of enzyme-mediated reactions.[15]

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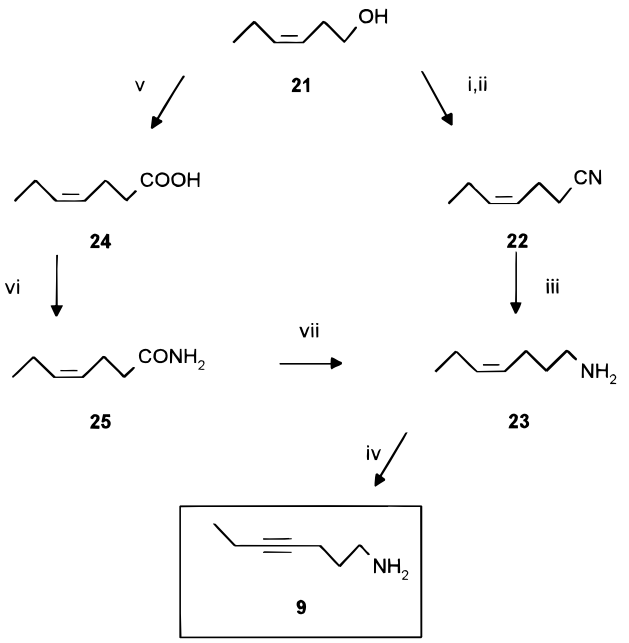
Scheme 3[a]



[a] Reagents: (i) $PPh_3/NBS/CH_2Cl_2$; (ii) $NaCN/H_2O/MeOH$; (iii) AlH_3/Et_2O; (iv) Ac_2O/pyridine; Br_2/CH_2Cl_2, and then KOH/MeOH, reflux; (v) $PPh_3/NBS/CH_2Cl_2$, Mg/THF/CO_2, and then H^+; (vi) $SOCl_2$, and then addition to 33% aqueous NH_3/THF; (vii) $LiAlH_4$/THF.

One of the major problems faced by the food flavor industry in the application of **1** and **2** in formulations is their stability. Indeed, freshly distilled **1** and **2** underwent rapid chemical decomposition, probably due to polymerization reactions. Conversely, both pyrrolines were stable in CH_2Cl_2 solutions for months. Recently it was found that the flavoring agent **1** remained stable for a long period in the solid phase and at low temperature after encapsulation in β-cyclodextrin.[10] Cyclodextrins are known to form stable inclusion complexes with a variety of compounds, with the guest molecule totally or partially allocated within the hydrophobic cavity of the host.[16] Thus, the improved chemical stability of **1** in the presence of β-cyclodextrin in the solid state may be due to the formation of supramolecular inclusion complexes. We have investigated the occurrence of this kind of complex between guest molecules like **1** and **2** and β-cyclodextrin by using NMR spectroscopy and mass spectrometry. The formation of an inclusion compound in water solution can be easily inferred from the values of the chemical shift of H-3′ and H-5′ of β-cyclodextrin. These protons are inside the cavity and are likely to undergo high field chemical shift variations due to the presence of the included guest molecule.[17] Conversely, no chemical shift changes are expected for all the others protons of the glucose units of β-cyclodextrin upon complexation. We have prepared water soluble complexes of both **1** and **2** with β-cyclodextrin (see Experimental Section for details). For the host−guest association of **1** and **2** we observed the high field variation of chemical shift of H-3′ and H-5′ only ($\Delta\delta$ of 0.04 ppm with respect to the reference uncomplexed β-cyclodextrin), proving that the interaction between the host and the guest is actually an inclusion.

The existence of supramolecular adducts between β-cyclodextrin and 2-acyl-1-pyrroline **1** and **2** was also checked in the gas phase using mass spectrometry. As recently reported,[18,19] soft ionization techniques, like FAB or electrospray, can be conveniently exploited for the investigation of host−guest complexes of β-cyclodextrin. A selection of data from FAB spectra of **1** and **2** in the presence of β-cyclodextrin (see Experimental Section for details on sample preparation and spectra acquisition) is reported in Table 1. The most striking feature coming out from the spectral data is the strong tendency of **1** and **2** to form supramolecular complexes with β-cyclodextrin and one molecule of matrix, 3-mercapto-1,2-propanediol (thioglycerol). The formation of ternary adducts is not uncommon in the field of cyclodextrin inclusion compounds, and some of them have been characterized.[20] A detailed study on gas-phase ternary complexes of β-cyclodextrin and acylpyrrolines is currently in progress and will be reported elsewhere. Tandem MS/MS experiments allowed us to gain insight on the molecular organization of the complexes generated by **1** and **2**. Collision-induced decomposition of the parent ion with m/z 1354, corresponding to the ternary complex **1**/β-cyclodextrin/thioglycerin protonated, afforded the daughter ions with m/z 220 (100% relative abundance) and m/z 202 (12), respectively, assigned to the adduct (**1**/thioglycerin) protonated and (**1**/thioglycerin) protonated with loss of one water molecule. Interestingly, no peak corresponding to protonated β-cyclodextrin was detectable, indicating that the parent ion is made of neutral β-cyclodextrin encapsulating the adduct **1** + thioglycerin + H^+.

Table 1. Summary of FAB-MS Spectral Data of Complexes of 1 and 2 with β-Cyclodextrin[a]

complex	[βCD + G + Mx + H]$^+$	[βCD + G + H]$^+$	[βCD + H]$^+$
1 + β-cyclodextrin	1354 (70)	1246 (5)	1135 (100)
2 + β-cyclodextrin	1368 (80)	1262 (12)	1135 (100)

[a] The columns report the m/z values and, in parentheses, the % relative abundance. Abbreviations: βCD: β-cyclodextrin; G: guest molecule, **1** or **2**; Mx: matrix molecule, 3-mercapto-1,2-propanediol.

NMR and MS experiments lead to the conclusion that compounds **1** and **2** are able to form inclusion complexes with β-cyclodextrin in water solution and in the gas phase. These findings also provide a possible rationale for the observed enhancement of stability of **1** in the solid state in the presence of β-cyclodextrin.[21]

Experimental Section

Uncorrected melting points were determined on a microstage block. TLC analyses were performed on Merck Kiesegel 60 F_{254} plates. All the chromatographic separations were carried out on silica gel columns. β-Cyclodextrin was provided by Roquette and used without any further purification. ^{1}H NMR spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise noted. The spectra of the inclusion complexes with cyclodextrin were recorded in D_2O solution at room temperature, with DSS external reference. Coupling constants are reported in hertz.

FAB-MS spectra were recorded on a triple quadrupole spectrometer, equipped with high voltage generator and using Xe as reactive gas. Collision-induced decomposition experi-

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ments were performed by using the first quadrupole for mass selection, the second as collision cell (Ar, $p = 0.8$ Torr), and the third as mass analyzer. The samples were prepared by dissolving into the matrix (3-mercapto-1,2-propandiol) commercial β-cyclodextrin and a CH_2Cl_2 solution of the acylpyrroline.

4-Hexyn-1-ol (17). Bromine (47 g, 0.298 mol) was added dropwise under stirring at 0 °C to a solution of 4-hexen-1-ol (**16**) (29.8 g, 0.298 mol) in CH_2Cl_2 (250 mL). The reaction mixture was kept for 2 h at rt and then evaporated under reduced pressure. The oily residue, 90 g, in EtOH (100 mL) was added slowly to a stirred solution of KOH (100 g, 1.6 mol) in EtOH (300 mL), and the reaction mixture was refluxed for 16 h. Concentrated HCl was then added at 0 °C to neutrality. The separated solid was filtered and washed with some ether. The organic phase was concentrated to a small volume, diluted with ice−water, and extracted with Et_2O (3 × 100 mL). The dried organic phase was carefully concentrated. Vacuum distillation (90 °C, water pump) of the residue afforded **17**, 23 g (79%). ^{1}H NMR ($CDCl_3$), δ: 1.65 (m, 2H), 1.75 (s, 1H), 2.13 (q, 2H, $J = 7.0$), 2.22 (s, 3H), 3.65 (q, 2H, $J = 7.0$). Anal. Calcd for $C_6H_{10}O$: C, 73.43; H, 10.27. Found: C, 73.47; H, 10.25.

4-Hexenoic Acid Amide (19). 3-Buten-2-ol (**18**) (200 mL, 2.32 mol), triethyl orthoacetate (426 mL, 2.32 mol), and acetic acid (7 mL) were refluxed for 32 h. The mixture was submitted to distillation discarding the 75−100 °C fraction and, subsequently, collecting the fraction boiling at 70−80 °C at 30 mmHg, 240 g, shown to be a *ca.* 65:35 mixture of ethyl 4-hexenoate and unreacted triethyl orthoacetate. The crude mixture in 250 mL of EtOH was refluxed 2 h with 680 mL of 10% NaOH. The reaction mixture was concentrated under reduced pressure and acidified with cold concd HCl while stirring with 250 mL of Et_2O. The aqueous phase was further extracted with ether. Evaporation of the organic extracts left a residue of 4-hexenoic acid, 164 g (62%). ^{1}H NMR ($CDCl_3$), δ: 1.65 (d, 3H, $J = 6.3$), 2.30−2.45 (m, 4H), 5.45 (m, 2H), 10.9 (s broad, 1H). The latter material neat was added dropwise under stirring to $SOCl_2$ (171 g, 1.44 mol), and the reaction mixture was refluxed for 4 h. The reaction mixture was submitted to vacuum distillation (40 mmHg) collecting at 85 °C the desired chloride, 137 g (72%). The latter product in THF (100 mL) was added dropwise to stirred 33% aqueous ammonia (500 mL). After 4 h the reaction mixture was extracted with Et_2O (3 × 150 mL). On crystallization from hexane of the residue obtained by evaporation of the dried organic phase was obtained the amide **19**, mp 98−100 °C, 97.5 g (86%). ^{1}H NMR ($CDCl_3$), δ: 1.65 (d, 3H, $J = 6.2$), 2.30 (m, 4H), 5.5 (m, 2H), 5.70 (s broad, 1H), 6.10 (s broad, 1H). Anal. Calcd for $C_6H_{11}NO$: C, 63.68; H, 9.80. Found: C, 63.60; H, 9.78.

1-Aminohex-4-ene (20). The amide **19** (52.5 g, 0.46 mol) in THF (100 mL) was added dropwise to a refluxing mixture of $LiAlH_4$ (28 g, 0.75 mol) in 300 mL of THF. After 6 h the reaction mixture was treated with AcOEt (500 mL) and a saturated solution of Na,K tartrate (150 mL). The dried organic phase was carefully evaporated to oily **20**, 23 g (50%). ^{1}H NMR ($CDCl_3$), δ: 1.2 (s, 2H), 1.5 (m, 2H), 1.65 (d, 3H, $J = 6.5$), 2.05 (m, 2H), 2.65 (t, 2H, $J = 6.7$), 5.4 (m, 2H). A solution of **20** (20 g, 0.2 mol) in CH_2Cl_2 (100 mL) was then treated at 0 °C with Ac_2O (40 mL) and pyridine (40 mL). After standing overnight, the reaction mixture was concentrated and the residue was partitioned between CH_2Cl_2 (200 mL) and ice−water. The organic phase was washed with $NaHCO_3$ solution, dilute HCl, and water. The residue obtained upon evaporation of the solvent was chromatographed on SiO_2 with hexane−AcOEt (55:45) to provide the acetyl derivative of the amine **20** (28.8 g, 88%), oil. ^{1}H NMR ($CDCl_3$), δ: 1.20 (s broad, 2H), 1.50 (m, 2H), 1.65 (d, 3H, $J = 7.0$), 2.05 (s, 3H), 2.70 (t, 2H, $J = 7.1$), 3.2 (q, 2H, $J = 7.1$), 5.35 (m, 2H). Anal. Calcd for $C_8H_{15}ON$: C, 68.04; H, 10.71. Found: C, 68.14; H, 10.66.

1-Aminohex-4-yne (8). Method A. To a stirred mixture of 4-hexyn-1-ol (**17**) (20 g, 0.204 mol) and Et_3N (31 mL, 0.226 mol) in CH_2Cl_2 (200 mL) was added 4-nitrobenzenesulfonyl chloride (50 g, 0.226 mol) in CH_2Cl_2 (100 mL) dropwise. After 24 h at rt, the reaction mixture was washed with water, dilute HCl, and $NaHCO_3$ solution. The residue obtained upon evaporation of the dried organic phase (90 g) was dissolved in dry ethanol and added to a 1 M solution of dry ammonia in EtOH (500 mL). After 2 h, the TLC analysis indicated the complete consumption of the starting material. The volume of the reaction mixture was reduced upon evaporation under vacuum in the cold. The oily material was taken up in 200 mL of CH_2Cl_2 and washed with dilute NaOH and water. The residue obtained upon evaporation of the solvent was distilled at 150 mmHg at 100 °C to provide **8**, oil, 11 g (55%). ^{1}H NMR ($CDCl_3$), δ: 1.35 (s broad, 2H), 1.60 (m, 2H), 1.78 (t, 3H, $J = 2.0$), 2.20 (m, 2H), 2.80 (t, 2H, $J = 7.0$).

Method B. To a stirred mixture of the acetyl derivative of 1-aminohex-4-ene **(20)** (see above) (36.9 g, 0.262 mol) in CH_2Cl_2 (150 mL) was added bromine (13.5 mL, 0.262 mol). After 2 h, the reaction mixture was concentrated, and the residue in EtOH was added to a stirred solution of KOH (40 g, 0.73 mol) in EtOH (200 mL). After 20 h reflux, the reaction mixture was concentrated under vacuum to a small volume at low temperature, diluted with water (150 mL), and extracted with CH_2Cl_2 (3 × 50 mL). Careful evaporation of the dried organic phase afforded an oily residue, providing upon distillation at 150 mmHg at 100 °C the desired amine **8**, 13 g (51%).

Nitrile of Hept-4-enoic Acid (22). *cis*-3-Hexen-1-ol (**21**) (100 g, 1 mol) in CH_2Cl_2 (1 L) at 0 °C was treated under stirring portionwise with Ph_3P (262 g, 1 mol) and NBS (178 g, 1 mol), keeping the temperature below 10 °C. After 20 h at rt, most of the solvent was evaporated, Et_2O/hexane 1:1 (500 mL) was added, and after standing overnight in the refrigerator, the precipitate was removed by filtration. The residue obtained upon evaporation of the organic phase was chromatographed on SiO_2 with CH_2Cl_2 to provide 1-bromohex-3-ene, oil, 120 g (73%). ^{1}H NMR ($CDCl_3$), δ: 0.95 (t, 3H, $J = 7.0$), 2.6 (q, 2H, $J = 7.0$), 3.35 (t, 2H, $J = 7.5$), 5.3 (m, 1H), 5.55 (m, 1H). A solution of the latter bromide (15 g, 92 mmol) in MeOH (30 mL) was added to NaCN (6.8 g, 130 mmol) in 30% aqueous methanol (100 mL). After 24 h, the reaction mixture was concentrated, diluted with ice−water, and extracted with Et_2O (2 × 50 mL). The residue obtained upon evaporation of the solvent afforded upon distillation (75 °C, 40 mmHg) the nitrile **22** , oil, 6.3 g (63%). ^{1}H NMR ($CDCl_3$), δ: 0.97 (t, 3H, $J = 7.5$), 2.1 (m, 2H), 2.35 (q, 2H, $J = 7.5$), 3.65 (t, 2H, $J = 6.9$), 5.32 (m, 1H), 5.57 (m, 1H). Anal. Calcd for $C_7H_{11}N$: C, 77.01; H, 10.16. Found: C, 77.09; H, 10.26.

1-Aminohept-4-ene (23). Method A. To $LiAlH_4$ (7.2 g, 0.2 mol) in Et_2O (150 mL) at −20 °C under stirring in N_2 atmosphere was added concd H_2SO_4 (10 g, 0.1 mol) during 3 h. To the resulting mixture was added dropwise the nitrile **22** (5.4 g, 0.05 mol), and the stirring was continued for 14 h. To the reaction mixture was added a small quantity of EtOH, followed by 10% NaOH (150 mL). Vacuum distillation (70−80 °C, water pump) of the organic residue affords the amine **23**. The material was contaminated by a few minor components which could not be separated. Thus the crude mixture was treated with Ac_2O, 10.2 g (0.1 mol), and pyridine, 8 g (0.1 mol), at rt for 16 h in CH_2Cl_2 (150 mL). The organic phase was treated with ice−water and washed with 3% $NaHCO_3$, 0.1 N HCl, and water. The residue obtained upon evaporation of the dried solution was chromatographed on SiO_2 with hexane−AcOEt (6:4) to provide 1-(*N*-acetylamino)hept-4-ene (4.7 g, 60%), oil. ^{1}H NMR ($CDCl_3$), δ: 0.95 (t, 3H, $J = 7.5$), 1.6 (m, 2H), 2.0 (s, 3H), 2.05 (m, 2H), 3.25 (q, 2H, $J = 7.5$), 5.3 (m, 2H), 5.9 (s broad, 1H). Anal. Calcd for $C_9H_{17}NO$: C, 69.63; H, 11.04. Found: C, 69.74; H, 10.96.

Method B. A Grignard reagent was prepared in THF (350 mL) from 1-bromo-*cis*-3-hexene (98 g, 0.6 mol) and Mg turnings (14.5 g, 0.6 mol). The consumption of Mg took place rapidly at gentle reflux. The resulting mixture was added dropwise to a large excess of solid CO_2 in THF (300 mL). After 2 h, an excess of cold concd HCl was added, and the mixture was concentrated to a small volume and then partitioned between water and Et_2O. The desired *cis*-4-heptenoic acid (**24**) was obtained in 83% yield upon extraction from the ether layer with 30% NaOH, acidification of the aqueous phase, and back extraction with Et_2O. ^{1}H NMR ($CDCl_3$), δ: 0.95 (t, 3H, $J = 7.5$), 2.05 (m, 2H), 2.4 (m, 4H), 5.30−5.55 (m, 2H). Product **24** (60 g, 0.47 mol) was added dropwise to $SOCl_2$ (41 mL, 0.55

mol) and then refluxed for 2 h. Vacuum distillation (80−85 °C at 30−40 mmHg) of the reaction mixture afforded the required acid chloride quantitatively. This material was added dropwise under stirring to 33% aqueous ammonia (100 mL) in THF (200 mL). The reaction mixture was concentrated under vacuum, and the residue was partitioned between water and CH_2Cl_2. The organic phase was washed with 3% $NaHCO_3$ and evaporated to dryness. The crystalline amide **25** separated from AcOEt−hexane, 9:1, mp 80−82 °C, in 77% yield. ^{1}H NMR ($CDCl_3$), δ: 0.95 (t, 3H, J = 7.5), 2.05 (m, 2H), 2.25−2.42 (m, 2H), 5.3−5.5 (m, 2H), 5.6 (s broad, 2H). The latter material was converted into the desired product **23** upon $LiAlH_4$ reduction, as reported for the conversion **19** and **20** in 41% yield.

1-Aminohept-4-yne (9). 1-(*N*-Acetylamino)hept-4-ene (46.5 g, 0.3 mol) in CH_2Cl_2 (200 mL) was treated with bromine (15.4 mL, 0.3 mol). After 2 h, the reaction mixture was evaporated under vacuum, and the oily residue was dissolved in EtOH (120 mL) and added under stirring to a solution of KOH (50 g, 1 mol) in EtOH (220 mL). After 24 h reflux, the reaction mixture was evaporated under vacuum at low temperature, and the residue was diluted with water (200 mL) and extracted with CH_2Cl_2 (3 × 70 mL). The oily residue obtained upon evaporation of the dried organic phase was distilled at 200 mmHg at 120−130 °C to provide the amine **9** (24.4 g, 73%). After distillation, **9** still contained some impurities, thus it was characterized as *N*-phenylacetyl derivative.

1-[*N*-(Phenylacetyl)amino]hex-4-yne (10) and 1-[*N*-(Phenylacetyl)amino]hept-4-yne (11). In parallel experiments, phenylacetyl chloride (8.9 mL, 67 mmol) was added to a solution of acetylenic amines (**8** and **9**, respectively) (45 mmol) in CH_2Cl_2 (100 mL) and triethylamine (12.5 mL, 90 mmol). After 24 h at rt, the organic phase was washed with dilute HCl, 3% $NaHCO_3$, and water. The residue of the dried organic phase was chromatographed on SiO_2 to afford, with AcOEt/hexane 1:1, the desired amides in *ca*. 50% yield. **10**, mp 87−89 °C, ^{1}H NMR ($CDCl_3$), δ: 1.61 (m, 2H), 1.72 (t, 3H, J = 2.0), 2.13 (m, 2H), 3.30 (q, 2H, J = 6.5), 3.60 (s, 2H), 5.54 (s broad, 1H), 7.2−7.5 (m, 5H). Anal. Calcd for $C_{14}H_{17}NO$: C, 78.10; H, 7.96. Found: C, 78.06; H, 8.01. **11**, mp 75−76 °C (hexane/AcOEt), ^{1}H NMR ($CDCl_3$), δ: 1.09 (t, 3H, J = 7.5), 1.60 (m, 2H), 2.10 (m, 4H), 3.29 (q, 2H, J = 7.5), 3.56 (s, 2H), 5.95 (s broad, 1H), 7.3 (m, 5H). Anal. Calcd for $C_{15}H_{19}NO$: C, 78.56; H, 8.35. Found: C, 78.76; H, 8.42.

1-[*N*-(Phenylacetyl)amino]-4,5-dioxohexane (12) and 1-[*N*-(Phenylacetyl)amino]-4,5-dioxoheptane (13). In parallel experiments, ozonized oxygen at −78 °C was bubbled through a solution of the acetylenic amides **10** and **11** (70 mmol) in CH_2Cl_2/MeOH 4:1 (80 mL). At the end of the reaction, monitored by the persistence of the blue color, N_2 was flushed for 10 min, followed by the addition of Me_2S (8.7 g, 140 mmol). After 4 h the reaction mixture was evaporated under vacuum. SiO_2 column chromatography of the residue affords, with hexane−AcOEt, the oily diketones in *ca*. 70% yield. ^{1}H NMR **12** ($CDCl_3$), δ: 1.76 (m, 2H), 2.30 (t, 2H, J = 7.2), 3.25 (q, 2H, J = 7.2), 3.57 (s, 2H), 3.62 (s, 3H), 5.58 (s broad, 1H), 7.2−7.4 (m, 5H). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 61.99; H, 6.93. Found: C, 61.87; H, 6.98. ^{1}H NMR **13** ($CDCl_3$), δ: 1.09 (t, 3H, J = 7.5), 1.72 (m, 2H), 2.73 (m, 4H), 3.21 (q, 2H, J = 7.5), 3.55 (s broad, 2 H), 3.55 (s, 2H), 5.58 (s, 1 H), 7.2−7.4 (m, 5 H). Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33. Found: C, 69.11; H, 7.29.

2-Acetyl-1-pyrroline (1) and 2-Propionyl-1-pyrroline (2). In parallel experiments, the amides **12** and **13** (typically, 3 g) in EtOH (5 mL) are added dropwise within 5 min to a mechanically stirred suspension of immobilized PGA (Recordati) (1.7 g, 600 ui/g (wet)) at 23 °C, while keeping pH 7.5 by addition of 0.1N NaOH. After 30 min the beads are decanted and the solution is extracted with Et_2O (5 × 20 mL). The organic phase is washed once with sat. NaCl soln. and dried on Na_2SO_4. The solvent was carefully evaporated through a short path column and the residue was distilled bulb-to-bulb at 30 mm/Hg (oven temp.: 90−100 °C) to provide products **1** and **2** in *ca*. 80% yield. ^{1}H NMR ($CDCl_3$) δ (**1**): 1.95 (tt, 2H, J = 7.5 and 8.4), 2.49 (s, 3H), 2.73 (tt, 2H, J = 8.4 and 2.5), 4.12 (tt, 2H, J = 7.5 and 2.5). (**2**): 1.12 (t, 3H, J = 7.3), 1.93 (tt, 2H, J = 8.5 and 7.5), 2.74 (tt, 2H, J = 8.5 and 2.5), 2.94 (q, 2H, J = 7.3), 4.11 (tt, 2H, J = 7.5 and 2.5).

Preparation of *β*-Cyclodextrin/Acylpyrroline Complex. General Procedure. Equimolar quantities of commercial β-cyclodextrin (βCD) and freshly distilled acylpyrroline were kneaded for 10 min and suspended in 1 mL of D_2O. Complete solubilization of the complex was obtained after heating in the case of **1** and after 15 min sonication at 50 °C in the case of **2**. βCD. ^{1}H NMR (D_2O) δ 5.05 (7H, d, H-1′, J = 3.8), 3.95 (7H, t, H-3′, J = 9.5), 3.86 (14H, m, H-6′), 3.84 (7H, m, H-5′), 3.63 (7H, dd, H-2′, J = 3.5 and 9.7), 3.57 (7H, t, H-4′, J = 9.4). Complex βCD/**1**. ^{1}H NMR (D_2O) δ: [βCD signals: 5.05 (7H, d, H-1′, J = 3.8), 3.91 (7H, t, H-3′, J = 9.5), 3.86 (14H, m, H-6′), 3.80 (7H, m, H-5′), 3.63 (7H, dd, H-2′, J = 3.5 and 9.7), 3.57 (7H, t, H-4′, J = 9.4); signals of **1**: 4.05 (2H, tt, J = 2.5 and 7.5), 2.73 (2H, tt, J = 2.5 and 8.2), 2.49 (3H, s), 1.95 (2H, tt, J = 7.5 and 8.2)]. Complex βCD/**2**. ^{1}H NMR (D_2O) δ: [βCD signals: 5.05 (7H, d, H-1′, J = 3.8), 3.91 (7H, t, H-3′, J = 9.5), 3.86 (14H, m, H-6′), 3.80 (7H, m, H-5′), 3.63 (7H, dd, H-2′, J = 3.5 and 9.7), 3.57 (7H, t, H-4′, J = 9.4); signals of **2**: 4.11 (2H, tt, J = 2.3 and 7.5), 2.94 (2H, q, J = 7.3), 2.74 (2H, tt, J = 2.5 and 8.0), 1.94 (2H, tt, J = 7.5 and 8.0), 1.12 (3H, t, J = 7.3)].

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