23, 129264-80-8; 24, 129264-81-9; 24 nitro alcohol derivative, 129285-44-5; 25, 116097-26-8; 26, 116180-69-9; 27, 129264-82-0; 28, 129264-83-1; 29, 129264-84-2; 30, 129264-85-3; 31, 61305-35-9; 32, 129264-86-4; 33, 129264-87-5; 34, 129264-88-6; 35, 129264-89-7; 36, 129264-90-0; 37, 129264-91-1; 38, 129264-92-2; 39, 129264-93-3; L-glutamic acid, 56-86-0; (4S)-4-[[(*tert*-butyldiphenylsilyl)oxy]-nethyl]-4-butanolide, 102717-29-3; dimethyl methylphosphonate, 756-79-6; crotonaldehyde, 4170-30-3; (phenylselenyl)acetic acid, 17893-46-8; nitromethane, 75-52-5; (S)-2-methylbutyric anhydride, 84131-91-9.

Supplementary Material Available: ^{1}H NMR spectra of all important intermediates (28 pages). Ordering information is given on any current masthead page.

Synthesis of 2,2-Dialkyl-1-aminocyclopropanecarboxylic Acids from α-Chloro Ketimines

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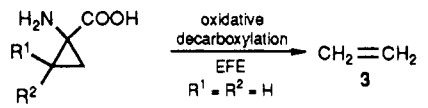
2,2-Dialkyl-1-aminocyclopropanecarboxylic acids, abbreviated as 2,2-dialkyl Acc's, are potential plant growth regulators which were synthesized by ring closure of α-chloro imines. The synthetic scheme leading to these Acc analogues entails a newly developed regiospecific synthesis of tertiary α-chloro ketimines, the trapping with cyanide of a transient Favorskii derived cyclopropylideneamine, and the hydrolytic conversion of 1-(*N*-*tert*-butylamino)-2,2-dialkylcyclopropanecarbonitriles into the title compounds.

Introduction

1-Aminocyclopropanecarboxylic acid (**1**, Acc) holds a prime position in plant physiology as it is oxidatively decarboxylated by the so-called "ethylene-forming enzyme" (EFE) to produce ethylene **3** (Scheme I).[1] Ethylene is a major plant hormone involved in regulating a number of important physiological processes including germination, ripening of fruits, abscission of fruits and leaves, and senescence. In recent years, the mechanism of the biochemical conversion of Acc to ethylene has received a great deal of attention. It started with the finding that the biosynthesis of ethylene occurs without exchange of the cyclopropane hydrogen atoms of Acc.[2] Detailed mechanistic studies by J. Baldwin and M. Pirrung have culminated in a proposal of the mechanism of the conversion of Acc to ethylene.[3-10] They suggested a stepwise enzymatic mechanism of cyclopropane ring opening in which stereochemical equilibration is faster than the subsequent bond breaking process.[6] It is proposed that this process occurs via a sequential single-electron-transfer pathway.[8,9] Also evidence has been obtained that the product of the ethylene biosynthesis from Acc, aside from carbon dioxide, is cyanide.[11,12] The physiological importance of Acc (**1**) and ethylene (**3**) has stimulated much efforts in the development of substrates that may induce an inhibition of the ethylene production, thus allowing a potential control of the ripening process. Initially, a great deal of interest was devoted to Acc analogues having a functionalized or derivatized nitrogen atom and a modified carboxylic acid functionality.[13-18] Some of these derivatives were patented for their plant growth regulating[13-15] and fungicidal properties,[16] while other Acc analogues stimulated fruit drop[17] or can be used as defoliants.[17,18] More recently, extensive efforts have been directed toward the synthesis of Acc analogues, monosubstituted at the cyclopropane ring in order to conserve the α-amino acid moiety.[19] Some

Scheme I



1: $R^1 = R^2 = H$ (Acc)
2: $R^1, R^2 \neq H$

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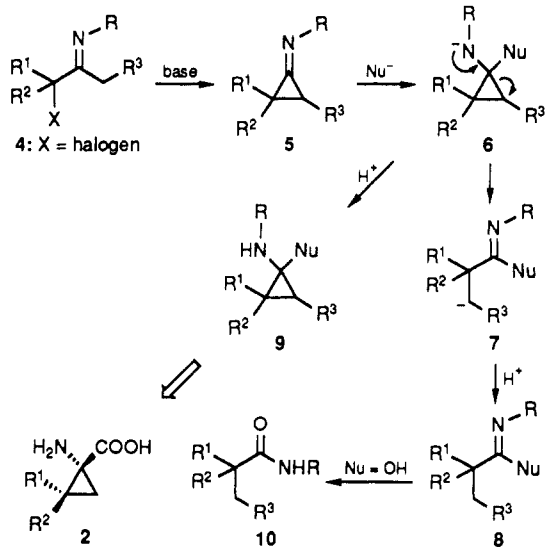
of these monoalkylated Acc derivatives showed an inhibition of the EFE and were substrates of this enzyme.[19g,i] The detailed mechanistic studies with 2-ethyl-1-aminocyclopropanecarboxylic acid,[20] with 2-methyl-1-aminocyclopropanecarboxylic acid,[19i] and with 2-cyclopropyl-1-aminocyclopropanecarboxylic acid,[19g] provided evidence concerning the steric requirements of the active center for ethylene production.

The synthesis of these monosubstituted Acc analogues can be divided into a few gross categories, including selective functional group transformations of one carboxylic ester moiety of cyclopropane-1,1-dicarboxylic esters, cyclopropanation with diazomethane of 4-alkylidene-5-(4*H*)-oxazolones or related dehydro amino acid derivatives, and dialkylation of protected glycine dianion equivalents. Up to now, the reported syntheses of monosubstituted Acc's via the above mentioned strategies did not allow the generation of geminal dialkylation at the cyclopropane moiety of Acc. However, it has been reported that ethyl 2-isocyano-3,3-dialkylacrylates undergo smooth cyclopropanation with dimethylsulfoxonium methylide but the resulting 1-isocyanocyclopropane-1-carboxylic esters were not further elaborated.[21] On the other hand, diphenyldiazomethane was reported to add to 2-arylideneoxazol-5-ones to afford the corresponding geminal diphenyl-substituted cyclopropanes.[22] Very recently, the synthesis of 1-amino-2,2-dimethylcyclopropanecarboxylic acid, i.e. 2,3-methanovaline, via addition of 2-diazopropane to a dehydroalanine derivative was disclosed.[23] This report unraveled an independent synthesis of the latter new α-amino acid, an alternative synthesis of which is described in the present article. Because of the fact that geminally dialkylated Acc analogues **2** have a potential plant growth regulating activity and that peptides containing them might be enzyme inhibitors,[23] the synthesis of these target molecules was performed starting from α-chloro imines **4** (X = Cl). In a preliminary paper, some highlights of the conversion of α-halo imines **4** into Acc analogues have been reported already.[24a] A more detailed description and additional information on this topic are disclosed here.

Scheme II



Results and Discussion

From previous studies on the reactivity of α-halo imines,[25] it was known that, under suitable reaction conditions, a base-induced 1,3-dehydrohalogenation of α-halo imines (**4**) could be performed leading to intermediate cyclopropylideneamines, **5**. Due to ring strain, these reactive Favorskii intermediates (**5**) readily undergo the addition of nucleophiles, e.g. alkoxides used as bases, to afford adducts **6**. Ring opening affords the most stable carbanion **7**, the protonated form of which represents the products **8** (Nu = OR) or **10** (Nu = OH) (Scheme II).[26] If the transient cyclopropylidenedeneamine (**5**) can be intercepted by a nucleophile, producing a stable adduct **9**, then those adducts can be elaborated for the construction of the desired 2,2-dialkyl-1-aminocyclopropanecarboxylic acids **2**. The transformation of the adduct **9** into the α-amino acids **2** requires the removal of the N-substituent R and the conversion of the nucleophilic moiety Nu into a carboxylic group (R^1 and R^2 are alkyl groups and R^3 = H). An obvious choice for the N-substituent was a benzyl group, but *N*-benzyl α-halo imines **4** (R = CH_2Ph), under basic conditions, gave rise to a variety of undesired side reactions.[25] As it was reasoned that cyanide would be a suitable trapping agent for cyclopropylideneamines and because the cyanide moiety can be transformed into a carboxylic group in acid medium, the *N*-*tert*-butyl substitution was evaluated as a way to make the desired α-amino acids. However, the aqueous acid medium not only could hydrolyze the nitrile and remove the *N*-*tert*-butyl substituent, it could also be harmful to the cyclopropane ring in terms of cleavage reactions. When putting together the requirements for adducts **9** and tracing it back to the starting α-halo imines **4**, it was necessary to synthesize *N*-*tert*-butyl tertiary α-halogenated methylketimines **4** (R = *t*-Bu; R^1, R^2 = alkyl; R^3 = H; X = halogen). Previous experience with α-bromo ketimines revealed their propensity for conversion into 1-aza 1,3-dienes by based-induced 1,2-dehydrobromination.[25] Since the Favorskii re-

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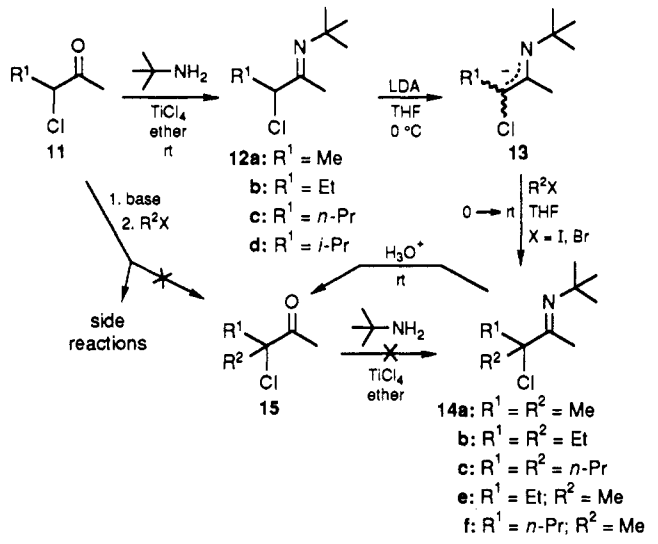
Table I. Cyanation of α-Chloro Ketimines 14 into 1-(*N*-*tert*-Butylamino)-2,2-dialkylcyclopropanecarbonitriles 18 and α-Cyanoaziridines 19

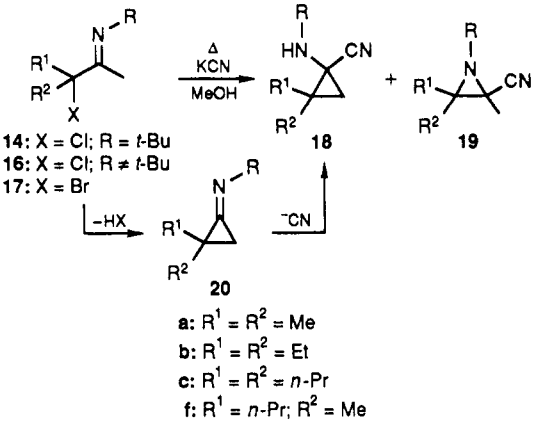
starting α-chloro imine	reaction conditions	cyclopropanes 18	aziridines 19	bp 18, °C (mmHg)
14a ($R^1 = R^2 = Me$)	2 equiv of KCN/MeOH Δ 5 h	47%	35%	86–89 (11)
14b ($R^1 = R^2 = Et$)	2 equiv of KCN/MeOH Δ 6 h	93%	<5%	74–80 (0.5)
14c ($R^1 = R^2 = n$-Pr)	2 equiv of KCN/MeOH Δ 6 h	92%	<6%	62–64 (0.05)
14f ($R^1 = n$-Pr; $R^2 = Me$)	2 equiv of KCN/MeOH Δ 6 h	90%[a]	<5%	-

[a] Mixture of cis and trans isomers (about 1:1).

Scheme III



Scheme IV



arrangement of α-bromo imines only occurs with specific substrates under appropriate reaction conditions,[26] no further attention was paid to α-bromo ketimines. The first synthetic experiments directed toward *N*-*tert*-butyl tertiary α-chlorinated methylketimines **14** (R = *t*-Bu; R^1, R^2 = alkyl; R^3 = H; X = Cl) met with great difficulties and disappointments. We found that in general, secondary α-chloro ketones **11** cannot be alkylated at the α-position to afford tertiary α-chloro ketones **15** by deprotonation at low temperature using a strong nonnucleophilic base and subsequent treatment of the enolate with an alkyl halide.[25] Also, the most general method for the synthesis of α-haloimines involving the condensation of an α-halo ketone with a primary amine in the presence of titanium(IV) chloride[27] is not applicable for the synthesis of *N*-*tert*-butyl tertiary α-chlorinated methylketimines **14**. Under a variety of conditions (temperatures, solvent, mode of addition, ...), no single product could be identified from the reaction of 3-chloro-3-methyl-2-butanone **15** ($R^1 = R^2 = Me$) with *tert*-butylamine in the presence of titanium(IV) chloride. Therefore, an alternative and flexible route to the desired α-chloro ketimines **14** was developed (Scheme III). Condensation of 3-chloro-2-alkanones **11**[28] with *tert*-butylamine in the presence of titanium(IV) chloride afforded the secondary α-chloro ketimines **12** in excellent yields (Scheme III). Regiospecific deprotonation of α-chloro ketimines **12** with lithium diisopropylamide in tetrahydrofuran and subsequent alkylation of the resulting 3-chloro-1-azaallylic anion **13** led to *N*-*tert*-butyl tertiary α-chloro ketimines **14** in excellent yields, free of any side product. If desired, α-chloro ketimines **14** can be hydrolyzed in aqueous acid to afford tertiary α-chloro ketones **15**, thus completing the synthesis from secondary α-chloro ketones **11** via α-chloro ketimines **12**.[29]

Having in hand the *N*-*tert*-butyl tertiary α-chloro ketimines **14**, we then studied cyanation of these substrates. Previous studies revealed that cyanation of α-halo imines under Favorskii conditions represented a useful reaction to form 1-(*N*-alkylamino)cyclopropane-1-carbonitriles **18**, but α-cyanoaziridine formation (see **19**) was the major reaction pathway (Scheme IV).[28,29] A detailed study of the cyanation of α-halo imines showed that the nature of the cyanide, the α-halogen, and the solvent played an important role, but that especially the introduction of steric bulk in the starting compound directed the reactivity toward cyclopropanes **18**.[28,29] The source of cyanide did not play an important role on the distribution of compounds **18** and **19** (a variety of cyanides was used, e.g. sodium, potassium, zinc, silver, copper, trimethylsilyl, and tetrabutylammonium cyanide).

Cyclopropane formation occurred in alcoholic medium but not in aprotic polar solvents such as acetonitrile, dimethyl sulfoxide, or dimethylformamide. α-Bromo ketimines **17** afforded α-cyanoaziridines **19**, accompanied by small amounts of α,β-unsaturated imines. Introduction of steric bulk at the α-position, i.e. increasing α,α-dialkylation, and at the nitrogen atom, i.e. *N*-*tert*-butyl substitution, increased dramatically the proportion of cyclopropanes at the expense of cyanoaziridines The results directed toward the desired cyclopropanation of α-chloro ketimines are summarized as in Table I. Reaction of *N*-*tert*-butyl α-chloro ketimines **14** ($R^1 = R^2$ = Me, Et, *n*-Pr; R = *t*-Bu) with potassium cyanide in methanol under reflux (5–6 h) gave rise to 1-(*N*-*tert*-butylamino)-2,2-dialkylcyclopropanecarbonitriles **18** ($R^1 = R^2$ = Me, 47%; $R^1 = R^2$ = Et, 93%; $R^1 = R^2$ = *n*-Pr, 92%; isolated yields are indicated) and, to a minor extent, α-cyanoaziridines **19**. Only the geminal dimethyl derivative **14** ($R^1 = R^2$ =

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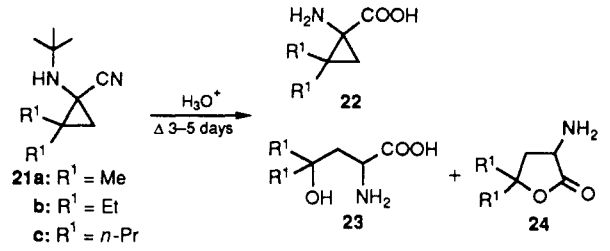
Table II. Synthesis of 2,2-Dialkyl-1-aminocyclopropanecarboxylic Acids 22, γ-Hydroxy-α-amino Acids 23, and α-Amino Lactones 24

starting compound	R^1	R^2	R	reaction conditions	% yield[a] 22	23	24
18a	Me	Me	*t*-Bu	10 equiv of 2 N HCl/H_2O Δ 3 days	40–60	20	–
18b	Et	Et	*t*-Bu	10 equiv of 6.6 N HCl/H_2O Δ 17.5 h	17	15	–
18b	Et	Et	*t*-Bu	10 equiv of 2 N HCl/H_2O Δ 5 days	10	17	24
18c	*n*-Pr	*n*-Pr	*t*-Bu	10 equiv of 5 N HCl/H_2O Δ 17 h	27	–	3
18c	*n*-Pr	*n*-Pr	*t*-Bu	10 equiv of 6 N HCl/H_2O Δ 3 days	–	–	28
22a	Me	Me	–	10 equiv of 5 N HCl/H_2O Δ 18.5 days	–	–	92
23a	Me	Me	–	Δ 180 °C (0.05 mmHg) (sublimation)[b]	–	26	56
22b	Et	Et	–	10 equiv of 6 N HCl/H_2O Δ 6–14 days	–	–	87

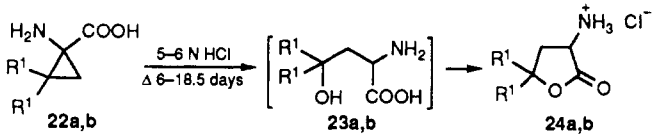
[a] Only pure isolated materials (after extensive chromatographic separation). [b] Partial decomposition occurs.

Scheme V

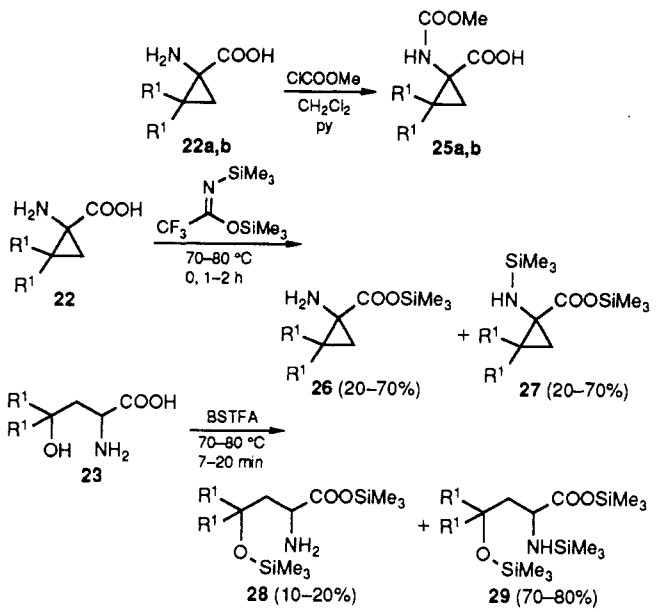


Me; R = *t*-Bu) gave rise to a 3:2 ratio of cyclopropanecarbonitrile **18** and α-cyanoaziridine **19**. The higher homologues **14** (R^1 = R^2 = Et, *n*-Pr) produced cyclopropanecarbonitriles **18** (92–93%) together with almost negligeable amounts of α-cyanoaziridines **19**. After purification by vacuum distillation, the 1-(*N*-*tert*-butylamino)-2,2-dialkylcyclopropanecarbonitriles **21** were treated with aqueous hydrogen chloride (2–5 N; 10 molar equiv) under reflux for 1–5 days to afford a reaction mixture containing the desired 2,2-dialkyl-1-aminocyclopropanecarboxylic acids **22** (17–60%), together with varying amounts of γ-hydroxy-α-amino acids **23** (0–26%) and α-amino-γ,γ-dialkyl-γ-butyrolactones **24** (0–24%) (Table II, Scheme V). The drastic hydrolytic procedure converted the nitrile function to the carboxylic acid and removed the *tert*-butyl substituent on nitrogen. Under these conditions, 1-amino-2,2-dimethylcyclopropanecarboxylic acid (**22a**) (R^1 = Me) was obtained in 40–60% yield, but an increasing geminal substitution (R^1 = Et, *n*-Pr) caused, not unexpectedly, the three-membered ring to open in a competitive reaction resulting in the formation of γ-hydroxy-α-amino acids **23**. The latter cyclized in the acid medium into α-amino lactones **24** upon proloned reflux. Accordingly, yields of the higher Acc analogues **22** dropped dramatically to 17–27%. The various reactions of the hydrolysis step, namely hydrolysis of the nitrile, deprotection on nitrogen, and cleavage of the cyclopropane ring, apparently, occur at competitive rates. However, monitoring of the reaction by thin-layer chromatography revealed that Acc analogues **22** are the initial reaction products, which are cleaved in the acid medium. This result is supported by the conversion of 1-amino-2,2-dimethylcyclopropanecarboxylic acid (**25a**) (R^1 = Me) with 5 N aqueous hydrogen chloride (10 molar equiv) under reflux for 18.5 days into α-amino-γ,γ-dimethyl-γ-butyrolactone (**24a**) (R^1 = Me) in 92% yield (isolated as the hydrochloride). In similar way, 2,2-diethyl-Acc **22b** was

Scheme VI



Scheme VII



transformed in aqueous acid under reflux into the corresponding α-amino-γ-butyrolactone **24b** in 80–87% yield (Scheme VI).

The reaction mixtures containing α-amino acids **22** and **23** and α-aminolactones **24** were separated by the usual chromatographic techniques (DOWEX 50X8-100; 1,2-epoxypropane treatment; gel filtration with Bio Gel P-2 200–400 mesh). The eluates were monitored by thin-layer chromatography using a 4:1:1 mixture of 1-butanol, acetic acid, and water. A ninhydrin solution and a copper(II) nitrate solution were used to visualize the spots and to stabilize them, respectively. Besides the usual spectroscopic analyses, including 360-MHz ^1H NMR, the novel Acc analogues were analyzed in derivatized form (Scheme VII).

Conversion of α-amino acids **22a,b** into carbamates **25a,b** was easily accomplished using methyl chloroformate

in dichloromethane in the presence of pyridine. Silylation of Acc analogues **22** with neat *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 70–80 °C afforded mono- and disilylated derivatives **26** and **27**, respectively, which are suitable for gas chromatographic analysis using capillary columns. Finally, Acc analogues **22** were analyzed on an amino acid analyzer (sulfonated polystyrene–divinylbenzene, particle size 6 μm, gradients of 0.2 and 1.0 N sodium citrate buffers).

Experimental Section

Gas chromatographic analyses were performed using preparative stainless steel columns (1.5 m 5–10% SE-30, Chromosorb W 60-80, H_2 carrier gas) or glass capillary columns (30 m, i.d. 0.5 mm, SE-30, He carrier gas).

Dry solvents were used in all experiments, e.g. ether (distilled from sodium wire), tetrahydrofuran (distilled from sodium benzophenone ketyl), dichloromethane (distilled from calcium hydride), and methanol (distilled from magnesium methylate). 3-Chloro-2-alkanones **11** were prepared from methyl acetoacetate via α-alkylation, α-chlorination, and methoxydecarbonylation as previously reported.[28] 3-Chloro-2-butanone is a commercial compound.

Synthesis of *N*-(3-Chloro-2-alkylidene)-*tert*-butylamines 12. A cooled (0 °C) and stirred solution of 0.2 mol of 3-chloro-2-alkanone **11** in 200 mL of dry ether (dried over calcium chloride), containing 0.8 mol of *tert*-butylamine, was treated dropwise with a solution of 0.12 mol of titanium(IV) chloride in 50 mL of pentane in such a way as to avoid a too vigorous reaction. The addition requires about 10–15 min. Stirring is continued at 0 °C during 15 min after which the reaction mixture is stirred at ambient temperature for 45 min. The reaction mixture is then poured rapidly in one portion into a separatory funnel, containing 500 mL of aqueous 0.5 N sodium hydroxide and 100 mL of ether. In order to avoid some hydrolysis of the α-chloro ketimine, it is absolutely necessary to shake immediately and vigorously the mixture in the separatory funnel. The ethereal layer was isolated, and the aqueous phase was extracted two times with ether. The combined ethereal extracts were dried with potassium carbonate for 5 min after which the drying agent was removed by filtration and replaced by fresh potassium carbonate. Drying is continued for 5–6 h or an overnight period. After filtration of the drying agent and evaporation of the solvent in vacuo, the remaining clear light yellow liquid consists of pure α-chloro ketimine **12**. Distillation in vacuo affords α-chloro ketimines **12**, free from any side product.

***N*-(3-Chloro-2-butylidene)-*tert*-butylamine, 12a** (R^1 = Me):[27] bp 57–59 °C (14 mmHg); yield 90%.

***N*-(3-Chloro-2-pentylidene)-*tert*-butylamine, 12b** (R^1 = Et): bp 74–76 °C (15 mmHg); yield 88%; IR (NaCl) 1672 cm^{-1} (C=N); ^1H NMR (CCl_4) δ 0.96 (3 H, t, J = 6.5 Hz, CH_2CH_3), 1.23 (9 H, s, Me_3), 1.77 (2 H, q, J = 6.5 Hz, CH_2CH_3), 1.94 (3 H, s, MeC=N), 4.07 (1 H, t, J = 6.5 Hz, CHCl); ^{13}C NMR ($CDCl_3$) δ 11.23 (q, CH_2CH_3), 15.53 (q, CH_3C=N), 29.01 (t, CH_2CH_3), 30.11 (q, Me_3), 55.11 (s, NC), 71.31 (d, CHCl), 163.51 (s, C=N); mass spectrum, m/z (%) 175/7 (M^+, 2), 160/2 (5), 147/9 (3), 140 (2), 127 (1), 120 (3), 113 (1), 104 (2), 98 (12), 84 (5), 57 (100), 56 (4), 55 (3), 42 (15), 41 (19), 40 (6). Anal. Found: N, 7.83. Calcd: N, 7.97.

***N*-(3-Chloro-2-hexylidene)-*tert*-butylamine, 12c** (R^1 = n-Pr): bp 86–88 °C (13 mmHg); yield 84%; IR (NaCl) 1669 cm^{-1} (C=N); ^1H NMR (CCl_4) δ 0.93 (3 H, ~t, J = 6 Hz, $(CH_2)_2CH_3$), 1–2 (4 H, m, $CH_2CH_2CH_2CH_3$), 1.23 (9 H, s, $(CH_3)_3$), 1.96 (3 H, s, CH_3C=N), 4.19 (1 H, t, J = 7 Hz, CHCl); ^{13}C NMR ($CDCl_3$) δ 13.58 (q, $(CH_2)_2CH_3$), 15.50 (q, CH_3, C=N), 19.97 (t, $CH_2CH_2CH_3$), 30.11 (q, $(CH_3)_3$), 37.75 (t, $CH_2CH_2CH_3$), 55.09 (s, NC), 69.55 (d, CHCl), 163.62 (s, C=N); mass spectrum, m/z (%) 189/91 (M^+, 1); 174/6 (6), 155 (2), 154 (2), 147/9 (14), 134 (3), 126 (3), 110 (2), 106 (2), 104 (5), 98 (19), 97 (2), 96 (2), 93 (2), 91 (6), 82 (3), 81 (3), 70 (3), 69 (2), 57 (100), 56 (5), 55 (5), 44 (2), 43 (7), 42 (15), 41 (19), 40 (14). Anal. Found: N, 7.49. Calcd: N, 7.38.

***N*-(3-Chloro-4-methyl-2-pentylidene)-*tert*-butylamine, 12d** (R^1 = i-Pr): bp 28–31 °C (0.05 mmHg) or 38–41 °C (0.1 mmHg); yield 45%; IR (NaCl) 1669 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 0.90 and 1.11 (2 × 3 H, d, J = 6.4 Hz, $CH(CH_3)_2$), 1.29 (9 H, s, $(CH_3)_3$), 1.8–2.2 (1 H, m, $CH(CH_3)_2$, 1.98 (3 H, m, CH_3C=N), 3.93 (1 H, d, J = 9.6 Hz, CHCl); ^{13}C NMR ($CDCl_3$) 15.68 (q, CH_3C=N), 19.71 and 20.26 (2 q, $CH(CH_3)_2$), 30.10 (q, $(CH_3)_3$), 33.22 (d, $CH(CH_3)_2$), 55.17 (s, NC), 76.63 (d, CHCl), 163.35 (s, C=N); mass spectrum, m/z (%) 189/91, (M^+, 1), 174/6 (5), 154 (7), 147/9 (8), 134 (3), 132 (3), 118 (3), 113 (3), 98 (18), 91 (6), 57 (100), 56 (5), 55 (5), 43 (5), 42 (16), 41 (19), 40 (1). Anal. Found: N, 7.45. Calcd: N, 7.38.

Synthesis of *N*-(3-Chloro-3-alkyl-2-alkylidene)-*tert*-butylamines 14. A cooled (0 °C) solution of 0.12 mol of lithium diisopropylamide (prepared from 0.12 mol of 2.5 M butyllithium in hexanes and 0.13 mol of diisopropylamine) in 120 mL of dry tetrahydrofuran under an inert atmosphere (nitrogen) was treated with 0.1 mol of secondary α-chloro ketimine **12**, dissolved in 15 mL of tetrahydrofuran. After stirring for 30 min at 0 °C, the solution of the 3-chloro-1-azaallyl anion **13** thus formed was treated with 0.11 mol of the alkyl halide R^2X (iodomethane, bromoethane, 1-bromopropane). Stirring was continued for 30 min at 0 °C and a further 90 min at ambient temperature. The reaction mixture was then poured into 300 mL of ice-cold 1 N sodium hydroxide. The organic layer was isolated, and the aqueous phase was extracted two times with ether. After drying of the combined extracts over potassium carbonate, filtration of the drying agent and evaporation of the solvent afforded the crude tertiary α-chloro ketimines **14** (purity >95%). Vacuum distillation provided the pure α-chloro ketimines **14** as colorless liquids.

***N*-(3-Chloro-3-methyl-2-butylidene)-*tert*-butylamine, 14a** (R^1 = R^2 = Me): bp 20–23 °C (0.2 mmHg); yield 90%; IR (NaCl) 1671 cm^{-1} (C=N); ^1H NMR (CCl_4) δ 1.24 (9 H, s, $(CH_3)_3$) 1.63 (6 H, s, $CCl(CH_3)_2$, 2.08 (3 H, s, CH_3C=N); ^{13}C NMR ($CDCl_3$) δ 15.92 (q, CH_3C=N), 30.03 (q, $(CH_3)_3$), 30.64 (2 q, $(CH_3)_2$), 54.64 (s, NC), 74.64 (s, CCl), 164.42 (s, C=N); mass spectrum, m/z (%) 175/7 (M^+, 0.3), 160/2 (4), 124 (2), 98 (20), 84 (13), 57 (100), 56 (3), 55 (3), 42 (13), 41 (23), 40 (5). Anal. Found: N, 7.86. Calcd: N, 7.97.

***N*-(3-Chloro-3-ethyl-2-pentylidene)-*tert*-butylamine, 14b** (R^1 = R^2 = Et): bp 96–100 °C (18 mmHg); yield 92%. Due to decomposition during vacuum distillation, α-chloro ketimine **14b** was used for further reactions without distillation (purity >95%): IR (NaCl) 1665 cm^{-1} (C=N); ^1H NMR (CCl_4) δ 0.85 (6 H, Me_2, t, J = 6.5 Hz, 2 × CH_2CH_3), 1.27 (9 H, s, $(CH_3)_3$), 1.7–2.2 (4 H, m, 2 × CH_2), 2.03 (3 H, s, CH_3C=N); ^{13}C NMR ($CDCl_3$) δ 9.15 (q, $(CH_2CH_3)_2$), 17.61 (q, CH_3C=N), 30.24 (q, $(CH_3)_3$), 32.59 (t, $(CH_2CH_3)_2$, 55.03 (s, NC), 83.62 (s, CCl), 163.51 (s, C=N); mass spectrum, m/z (%) 203/5 (M^+, 0.7), 183/5 (2), 175/7 (2), 168 (4), 167 (2), 152 (2), 119 (1), 113 (3), 112 (10), 111 (1), 109 (2), 104 (1), 99 (1), 98 (18), 96 (5), 83 (1), 82 (2), 69 (2), 67 (2), 57 (100), 56 (3), 55 (2), 43 (2), 42 (11), 41 (15), 40 (5). Anal. Found: N, 7.01. Calcd: N, 6.87.

***N*-(3-Chloro-3-propyl-2-hexylidene)-*tert*-butylamine, 14c** (R^1 = R^2 = n-Pr): bp 45–49 °C (0.05 mmHg); yield 93%. Compound **14c** can also be used as the crude product without vacuum distillation (purity >95%): IR (NaCl) 1668 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 0.90 (2 × 3 H, ~t, J = 6 Hz, $(CH_2)_2CH_3$), 1.1–1.7 (2 × 2 H, m, $CH_2CH_2CH_2CH_3$), 1.26 (9 H, s, $(CH_3)_3$), 1.8–2.1 (2 × 2 H, m, $CH_2CH_2CH_2CH_3$), 2.03 (3 H, s, CH_3C=N); ^{13}C NMR ($CDCl_3$) δ 14.41 (q, $(CH_2)CH_3$), 17.49 (q, CH_3C=N), 18.16 (t, $CH_2CH_2CH_3$), 30.24 (q, $(CH_3)_3$), 42.73 (t, $CH_2CH_2CH_3$), 54.96 (s, NC), 82.27 (s, CCl), 163.74 (s, C=N); mass spectrum, m/z (%) 232/4 (M^+ + 1, 0.2), 231/3 (M^+, 0.2), 216/8 (2), 196 (4), 195/7 (2), 189/91 (12), 180 (2), 166 (2), 160/2 (4), 140 (8), 138 (2), 133 (2), 124 (4), 110 (10), 104 (5), 98 (21), 96 (3), 82 (2), 81 (2), 70 (2), 69 (2), 67 (2), 57 (100), 56 (4), 55 (10), 53 (3), 43 (5), 42 (13), 41 (21), 40 (2). Anal. Found: N, 6.17. Calcd: N, 6.04.

***N*-(3-Chloro-3-methyl-2-pentylidene)-*tert*-butylamine, 14e** (R^1 = Et; R^2 = Me): bp 79–81 °C (15 mmHg); yield 81%; IR (NaCl) 1670 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 0.90 (3 H, t, J = 7 Hz, CH_2CH_3), 1.27 (9 H, s, $(CH_3)_3$), 1.60 (3 H, s, CH_3CCl), 1.99 (2 H, q, J = 7 Hz, CH_2CH_3), 2.06 (3 H, s, CH_3C=N); ^{13}C NMR ($CDCl_3$) δ 9.54 (q, CH_2CH_3), 16.40 (q, CH_3C=N), 27.52 (q, $CClCH_3$), 30.15 (q, $(CH_3)_3$), 35.43 (t, CH_2), 54.86 (s, NC), 78.66 (s, CCl), 163.94 (s, C=N); mass spectrum, m/z (%) 189/91 (M^+, 2), 174/6 (4), 161/3 (2), 154 (7), 138 (2), 113 (2), 98 (39), 82 (3), 57 (100), 42 (12), 41 (16), 40 (3). Anal. Found: N, 7.42. Calcd: N, 7.38.

***N*-(3-Chloro-3-methyl-2-hexylidene)-*tert*-butylamine, 14f** (R^1 = n-Pr; R^2 = Me): bp 90–92 °C (15 mmHg); yield 90% (partial

decomposition upon vacuum distillation); IR (NaCl) 1670 cm^{-1} (C=N); ^1H NMR ($CDCl_3$) δ 0.90 (3 H, ~t, $(CH_2)_2CH_3$), 1-2 (4 H, m, $CH_2CH_2CH_3$), 1.26 (9 H, s, $(CH_3)_3$), 1.63 (3 H, s, $CClCH_3$), 2.07 (3 H, s, CH_3C=N); ^{13}C NMR ($CDCl_3$) δ 14.35 (q, $(CH_2)_2CH_3$), 16.37 (q, CH_3C=N), 18.54 (t, $CH_2CH_2CH_3$), 28.07 (q, $CClCH_3$), 30.14 (q, $(CH_3)_3$), 44.94 (t, $CH_2CH_2CH_3$), 54.84 (s, NC), 78.18 (s, CCl), 164.00 (s, C=N); mass spectrum, m/z (%) 203/5 (M^+ + 1, 0.1), 203/5 (M^+, 0.2), 188/90 (3), 168 (3), 161/3 (11), 152 (2), 112 (9), 110 (2), 105/7 (5), 98 (19), 96 (4), 70 (2), 69 (3), 68 (2), 67 (2), 57 (100), 56 (4), 55 (5), 53 (3), 42 (14), 41 (20). Anal. Found: N, 6.80. Calcd: N, 6.87.

Synthesis of 1-(*N*-*tert*-Butylamino)-2,2-dialkylcyclopropanecarbonitriles, 18 (R = *t*-Bu). A 10% (w/v) solution of 0.1 mol of *N*-*tert*-butyl tertiary α-chloro ketimine **14** in dry methanol was treated with 0.2 mol of potassium cyanide. After 5–6 h of stirring under reflux, half of the methanol was evaporated in vacuo. The residual half was poured into 300 mL of aqueous 0.5 N sodium hydroxide. The mixture was extracted three times with dichloromethane, and the combined extracts were dried ($MgSO_4$), filtered, and evaporated to afford a reaction mixture containing 1-(*N*-*tert*-butylamino)-2,2-dialkylcyclopropanecarbonitriles **18** and 1-*tert*-butyl-2-methyl-3,3-dialkylaziridine-2-carbonitriles **19**. The cyclopropanecarbonitriles **18** were purified by vacuum distillation. Only in the case of the geminal dimethyl substitution (cf. **18a** and **19a**), the vacuum distillation was performed via a Vigreux column of 10 cm.

1-(*N*-*tert*-Butylamino)-2,2-dimethylcyclopropane-1-carbonitrile, 21a (R = *t*-Bu; R^1 = Me): bp 86–89 °C (11 mmHg); yield 46–56%; IR (NaCl) 2219 (C≡N), 3332 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 0.87 and 1.11 (2 H, AB, dd, J = 5.1 Hz, CH_2); 1.18 and 1.27 (each 3 H, 2 s, overlap, Me_2), 1.23 (9 H, s, Me_3); ^{13}C NMR ($CDCl_3$) δ 19.49 and 23.22 (2 q, Me_2), 26.06 (s, CMe_2), 30.12 (q, $C(CH_3)_3$, 30.39 (t, CH_2), 33.01 (s, NCCNH), 52.21 (s, $C(CH_3)_3$), 123.41 (s, C≡N); mass spectrum, m/z (%) 166 (M^+, 5), 151 (9), 110 (20), 109 (9), 95 (94), 82 (9), 68 (8), 57 (100), 56 (13), 55 (9), 42 (11), 41 (52). Anal. Found: N, 16.93. Calcd: N, 16.85.

1-*tert*-Butyl-2,3,3-trimethylaziridine-1-carbonitrile, 19a (R = *t*-Bu; R^1 = Me): This compound showed spectroscopic data identical with that of the known compound[32] and revealed data similar to many analogues prepared previously.[30,31]

1-(*N*-*tert*-Butylamino)-2,2-diethylcyclopropane-1-carbonitrile, 21b (R = *t*-Bu; R^1 = Et): bp 74–80 °C (0.5 mmHg); yield 93%; IR (NaCl) 2220 (C≡N), 3335 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 0.7–1.2 (m, 8 H, 2 Me and CH_2-ring), 1.23 (9 H, s, $(CH_3)_3$), 1.4–1.8 (m, 4 H, 2 CH_2Me); ^{13}C NMR ($CDCl_3$)δ 10.06 and 10.84 (2 q, $(CH_3CH_2)_2$, 21.05 and 26.29 (2 t, $(CH_3CH_2)_2$), 29.35 (t, CH_2-ring), 30.28 (q, $C(CH_3)_3$, 33.50 (s, $C(CH_2CH_3)_2$), 35.67 (s, NCCNH), 52.63 (s, $C(CH_3)_3$, 123.73 (s, C≡N); mass spectrum, m/z (%) 194 (M^+, 5), 179 (8), 165 (12), 137 (5), 123 (4), 109 (100), 96 (4), 82 (8), 70 (11), 69 (14), 68 (11), 57 (89), 56 (9), 55 (17), 42 (17), 41 (56). Anal. Found: N, 14.29. Calcd: N, 14.42.

1-(*N*-*tert*-Butylamino)-2,2-dicyclopropane-1-carbonitrile, 21c (R = *t*-Bu; R^1 = *n*-Pr): bp 62–64 °C (0.05 mmHg); yield 92%; IR (NaCl) 2220 (C≡N), 3330 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 0.8–1.70 (m, all H except *t*-Bu), 1.24 (s, 9 H, *t*-Bu); ^{13}C NMR ($CDCl_3$) δ 14.34 (q, $(CH_3(CH_2)_2)_2$), 19.21 and 19.82 (2 t, $(CH_3CH_2CH_2)_2$), 29.71 (t, CH_2-ring), 30.30 (q, $C(CH_3)_3$), 31.00 and 36.14 (t, $(CH_3CH_2CH_2)_2$, 33.03 or 33.58 (s, $(CH_3(CH_2)_2)_2C$), 33.58 or 33.03 (s, CNH), 52.65 (s, $C(CH_3)_3$), 123.77 (s, C≡N); mass spectrum, m/z (%) 222 (M^+, 2), 207 (12), 179 (11), 165 (5), 137 (9), 123 (71), 109 (8), 96 (6),95 (6), 81 (28), 70 (7), 69 (17), 68 (12), 57 (100), 56 (18), 55 (23), 43 (8), 42 (14), 41 (55). Anal. Found: N, 12.49. Calcd: N, 12.60.

1-(*N*-*tert*-Butylamino)-2-methyl-2-propylcyclopropane-1-carbonitrile, 18f (R = *t*-Bu; R^1 = Me; R^2 = *n*-Pr): crude yield 90%; mixture of cis and trans isomers (about 1:1); IR (NaCl) 2219 (C=N), 3330 cm^{-1} (NH); ^1H NMR ($CDCl_3$) δ 0.7–1.6 (m, all H except Me_2 and *t*-Bu), 1.24 and 1.26 (2 × 9 H, 2 s, $(CH_3)_3$), 1.42 and 1.51 (2 s, 2 × 3 H, 2 CH_3), cis/trans ~1/1; ^{13}C NMR ($CDCl_3$) δ 14.23, 14.32, 16.58, 19.61, 20.13, 20.64, 29.56, 29.84, 30.21 (Me_3), 32.87, 33.40, 34.70, 39.35, 52.36, 52.45, 123.53, 123.63 (3 signals overlap); mass spectrum, m/z (%) 194 (M^+, 3), 179 (12), 151 (7), 138 (3), 137 (4), 123 (6), 110 (5), 109 (11), 96 (9), 95 (86), 84 (2), 82 (4), 81 (5), 70 (12), 69 (12), 68 (13), 57 (100), 56 (11), 55 (13), 53 (6), 43 (5), 42 (15), 41 (52). Anal. Found: N, 14.31. Calcd: N, 14.42.

Acidic Hydrolysis of 1-(*N*-*tert*-Butylamino)-2,2-dialkylcyclopropane-1-carbonitriles 21 to Acc Analogues 22. A mixture of 0.05 mol of 1-(*N*-*tert*-butylamino)-2,2-dialkylcyclopropane-1-carbonitrile **21** and 0.5 mol of 5 N aqueous hydrogen chloride was refluxed for the time indicated in Table II. The hydrolysis was monitored by IR analysis (disappearance of the nitrile band). After cooling, the reaction mixture was extracted with dichloromethane in order to remove organic impurities. The aqueous phase was evaporated to dryness in vacuo. The solid residue was dissolved in 20 mL of distilled water and evaporated again to dryness in vacuo. The remaining solid was dissolved in 15 mL of distilled water, and the mixture was loaded on a Dowex 50X8-100 cationic exchange column (100 g, acid form). Three portions of 40 mL of distilled water were passed through the column. Afterward, elution was performed with concentrated aqueous ammonia. Fractions of 30–40 mL were collected. The first fractions contained mainly Acc analogues **22**, in addition to ammonium chloride. Further elution with concentrated ammonia afforded fractions containing γ-hydroxy-α-amino acids **23**. The α-amino lactones **24** eluted in the last fractions and were only isolated for experiments performed for an extended period of time. The eluates were monitored by means of thin-layer chromatography using silica gel 60 F_{254} plates (5 × 10 cm; thickness 0.25 mm). The solvent combination of 1-butanol–acetic acid–water (ratio 4:1:1) revealed the best separation of ninhydrin-positive spots. The ninhydrin reagent was prepared from 0.3 g of ninhydrin in 100 mL of 1-butanol, containing 3 mL of acetic acid. The stabilization of the spots could be enhanced by spraying with a solution prepared from 100 mL of ethanol, 1 mL of saturated aqueous copper(II) nitrate, and 0.2 mL of 10% nitric acid. The loading of the Dowex 50X8-100 column in proton form was performed by washing the weighed amount of resin in the column with several portions of distilled water, then several washings with 2 N hydrogen chloride until acidic, followed by washing with 2 N sodium hydroxide until alkaline, and finally a washing with 2 N hydrogen chloride until acidic. The regeneration of the Dowex 50 column was executed with 2 N or 4 N hydrogen chloride and a final washing with distilled water until neutral.

Additional purification of the evaporated fractions of the eluates was performed by crystallization in methanol–ether–water (5:2:1). Alternatively, these fractions (850 mg) could be refluxed with a mixture of 1,2-epoxypropane (5 mL) and absolute ethanol (15 mL). After evaporation of half of the solvent, crystallization at −20 °C afforded mainly the ring opening products **23**. Besides these crystallizations, chromatographic separations were executed under conditions which were less drastic than those of the prepurification step using the large pH jump. To this end, gradient elution with aqueous hydrogen chloride (0–2 N) was done. Fractions of 40 mL were collected and monitored with ninhydrin (extinction determination at 570 nm). The ninhydrin solution was prepared by mixing 0.8 g of $SnCl_2$(aq), 500 mL of citrate buffer (0.2 M citrate buffer, pH 5), 20 g of ninhydrin, and 500 mL of methyl cellosolve.

An alternative separation (200 mg product) was executed on a Dowex 50 column using an ammonium hydroxide gradient (0–1 N). Fractions of 30 mL were collected. Less good separations were obtained with this technique. The separation of the mixture of compounds **22** and **23** was also verified by gel filtration. A mixture of reaction products **22** and **23**, obtained from refluxing cyclopropanecarbonitrile **21b** (R^1 = Et) with 10 equiv of 6.6 N aqueous HCl for 17.5 h, was dissolved in 1 mL of distilled water. After loading on a Bio Gel column (53 g Bio Gel P-2, 200–400 mesh) with an effective length of 118 cm and internal diameter 14 mm, the elution was performed with 0.3 M aqueous acetic acid. Fractions of 2.75 mL were collected every 10 min. Fraction 49 up to 53 contained γ-hydroxy-α-amino acid **23b** (R^1 = Et) (8 mg), while fractions 56–67 contained pure Acc analogue **22b** (R^1 = Et) (9 mg).

α-Amino acid analyses were executed with a Varian 5060 liquid chromatograph and a Varian Vista CDS 401 integrator. The same mixture (1 mg) of **22b** and **23b**, obtained as in the last

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paragraph, was dissolved in 10 mL of 0.2 N sodium citrate buffer pH 2.75; 10 μL of this solution was injected on a Micropack column (length 15 cm, internal diameter 0.41 cm, packed with sulfonated polystyrene-divinylbenzene, particle size 6 μm). A gradient elution with two sodium citrate buffers of pH 2.75 (0.2 N) and pH 7.4 (1.0 N) was used at a temperature of 60 °C and a flow rate of 0.3 mL/min (ninhydrin detection at 570 nm). Acc analogue **22b** (R^1 = Et) showed a retention time of 45.9 min, while α-amino acid **23b** eluted after 35.8 min.

The relative retention times of Acc analogues **22** were compared with a number of α-amino acids and were found to obey the following order of elution (cation exchange column): proline < glycine < alanine < cysteine < valine < methionine < isoleucine < leucine < norleucine < **22a** < tyrosine < phenylalanine < **22b** < lysine < histidine < arginine.

Methoxycarbonylation of Acc Analogues. A mixture of 150 mg (0.001 mol) of Acc analogue **22b** (R^1 = Et) and 0.0011 mol of pyridine in 10 mL of dichloromethane was treated dropwise at 0 °C with 0.0011 mol of methyl chloroformate. After stirring for 3 h at room temperature, the reaction mixture was poured into 30 mL of water, extracted with dichloromethane, dried ($MgSO_4$), and evaporated in vacuo. The residue was crystallized from 10 mL of pentane at −20 °C to afford 1-(*N*-(methoxycarbonyl)-amino)-2,2-diethylcyclopropane-1-carboxylic acid, **25b** (63% yield).

2,2-Diethyl-1-(*N*-(methoxycarbonyl)amino)cyclopropanecarboxylic acid, 25b: mp 136 °C; IR (KBr) 3290 (NH), 1720 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 0.80 and 0.97 (6 H, 2 t, *J* = 6 Hz, 2 Me), 1.4–1.9 (4 H, m, 2 CH_2), 3.70 (3 H, s, OMe), CH_2-ring protons covered by both methyl signals, 5.6 (1 H, s, br, N*H*), COO*H* invisible; ^{13}C NMR ($CDCl_3$) δ 10.43 and 10.73 (2 q, 2 *Me*CH_2), 21.44 and 23.55 (2 t, 2 Me*C*H_2), 28.20 (t, CH_2-ring), 39.57 (s, CEt_2), 43.59 (s, *C*-NH), 52.59 (q, OCH_3), 158.12 (s, *C*OOMe), 177.24 (s, *C*OOH); mass spectrum, *m/z* (%) (only abundancies above 10%, except the molecular ion, are given) 215 (M^+, 6), 197 (61), 182 (56), 168 (59), 154 (26), 142 (9), 140 (26), 138 (12), 136 (39), 128 (16), 127 (16), 122 (13), 110 (62), 108 (14), 100 (11), 96 (14), 95 (20), 94 (42), 83 (14), 82 (32), 81 (12), 79 (19), 76 (23), 70 (26), 69 (26), 68 (15), 67 (18), 59 (45), 57 (19), 56 (32), 55 (92), 54 (14), 53 (18), 45 (14), 44 (12), 43 (33), 42 (73), 41 (100). Anal. Found: N, 6.38. Calcd: N, 6.51.

2,2-Dimethyl-1-(*N*-(methoxycarbonyl)amino)cyclopropanecarboxylic acid, 25a: mp 148 °C; IR (KBr) 3290 (NH), 1710 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.02 and 1.22 (each 1 H, AB, *J* = 5.6 Hz, CH_2-ring), 1.25 (6 H, s, 2 Me), 3.67 (3 H, s, OMe), 5.4 (1 H, s, br, N*H*), COO*H* invisible; ^{13}C NMR ($CDCl_3$) δ 19.53 and 21.81 (2 q, 2 Me), 30.66 (t, CH_2-ring), 31.63 (s, *C*Me_2), 43.75 (s, *C*NH), 52.51 (q, OMe), 157.29 (s, *C*OOMe), 168.25 (s, *C*OOH); mass spectrum, *m/z* (%) 187 (M^+, 22), 170 (70), 169 (54), 142 (100), 141 (18), 137 (16), 110 (44), 99 (14), 82 (76), 69 (16), 68 (13), 67 (19), 59 (24), 57 (29), 56 (23), 55 (25), 43 (54), 42 (60), 41 (62). Anal. Found: N, 7.35. Calcd: N, 7.48.

Silylation of Acc Derivatives with BSTFA. A mixture of 3 mg of 2,2-dimethyl-1-aminocyclopropanecarboxylic acid (**22a**) in 120 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was heated at 70 °C in a closed vial during 30 min. After cooling, 2–3 drops of pentane were added, and the reaction mixture was analyzed directly by GC–MS (capillary column). Prolonged heating resulted in increased disilylation (compound **27**). This silylation method is also applicable for the derivatization and analysis of γ-hydroxy-α-amino acids **23**. Mass spectral data (via GC–MS analysis) are given below.

Compound 26a (R^1 = Me): *m/z* (%) 201 (M^+, 14), 186 (27), 111 (36), 96 (36), 84 (28), 83 (100), 82 (18), 75 (7), 74 (38), 73 (19), 68 (45), 57 (8), 56 (9), 55 (9), 47 (10), 45 (20), 43 (19), 42 (18), 41 (19).

Compound 26b (R^1 = Et): *m/z* (%) 229 (M^+, 15), 214 (12), 201 (6), 200 (35), 144 (14), 139 (22), 124 (6), 116 (9), 112 (9), 111 (10), 110 (90), 96 (12), 82 (100), 75 (46), 74 (14), 73 (58), 69 (7), 55 (15), 47 (8), 45 (18), 43 (12), 42 (15), 41 (15).

Compound **27a** (R^1 = Me): *m/z* (%) 273 (M^+, 11), 258 (4), 230 (4), 186 (6), 156 (20), 155 (10), 147 (33), 140 (10), 111 (4), 100 (6), 96 (7), 84 (4), 83 (17), 82 (12), 81 (4), 77 (4), 75 (23), 73 (100), 72 (4), 68 (6), 59 (5), 47 (4), 45 (23), 44 (7), 43 (7), 42 (4), 41 (6).

Compound **27b** (R^1 = Et): *m/z* (%) 301 (M^+, 5), 272 (5), 196 (5), 184 (16), 182 (7), 184 (14), 142 (15), 110 (8), 100 (6), 82 (6), 77 (6), 75 (14), 73 (100), 45 (15), 40 (5).

Compound **28a** (R^1 = Me): *m/z* (%) 291 (M^+, 0.3), 131 (100, $Me_2C{=}O^+SiMe_3$).

Compound **28b** (R^1 = Et): *m/z* (%) 319 (M^+, 0.1), 159 (100, $Et_2C{=}O^+SiMe_3$).

Compound **29a** (R^1 = Me): *m/z* (%) 363 (M^+, 0.3), 131 (100, $Me_2C{=}O^+SiMe_3$).

Compound **29b** (R^1 = Et): *m/z* (%) 391 (M^+, 1), 159 (100, $Et_2C{=}O^+SiMe_3$).

Spectrometric Data of 2,2-Dialkyl-1-aminocyclopropanecarboxylic Acids 22, γ-Hydroxy-α-amino Acids 23, and α-Amino Lactones 24 (Hydrochlorides). 2,2-Dimethyl-1-aminocyclopropanecarboxylic acid, 22a (see ref 23): mp >260 °C; IR (KBr) 2300–3600 (OH and NH_2), 1540–1650 cm^{-1} (C=O); ^1H NMR (D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.93 (1 H, AB, J_{ab} = 6.4 Hz, C*H*-ring), 1.11 and 1.14 (6 H, 2 s, 2 Me), 1.29 (1 H, AB, J_{ab} = 6.4 Hz, C*H*-ring); ^{13}C NMR (D_2O; ref 1,4-dioxane δ 67.4 ppm) δ 20.20 and 21.15 (2 q, 2 Me), 23.82 (s, *C*Me_2), 24.40 (t, CH_2-ring), 45.66 (s, *C*NH_2), 174.97 (s, *C*OOH). Anal. Found: N, 10.70. Calcd: N, 10.84.

2,2-Diethyl-1-aminocyclopropanecarboxylic acid, 22b: mp >260 °C (very light sublimation from 190 °C and some brown coloring at 250 °C); IR (KBr) 2400–3600 (OH and NH_2), 1520–1640 cm^{-1} (C=O); ^1H NMR (360 MHz) (DCl–D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.74 (3 H, t, *J* = 7.4 Hz, Me), 0.94 (3 H, t, *J* = 7.3 Hz, Me), 1.18 (1 H, qd, *J* = 7.3 Hz and *J* = 14.6 Hz, HC*H*), 1.20 (1 H, d, *J* = 6.7 Hz, C*H*-ring), 1.40 (1 H, qd, *J* = 7.4 and 14.8 Hz, HC*H*), 1.52 (1 H, d, J_{ef} = 6.7 Hz, CH_a), 1.73 (2 H, 2 qd, *J* = 7.4 Hz and *J* = 15.0 Hz, 2 HC*H*); ^{13}C NMR (DCl–D_2O; ref 1,4-dioxane = δ 67.4 ppm) δ 10.59 and 10.88 (2 q, (*Me*CH_2)$_2$), 22.16 and 23.21 (2 t, (Me*C*H_2)$_2$), 25.24 (t, *C*H_2-ring), 37.55 (s, *C*Et_2), 43.82 (s, *C*NH_2), 172.41 (s, *C*OOH). Anal. Found: N, 8.96. Calcd: N, 8.91.

2,2-Di-*n*-propyl-1-aminocyclopropanecarboxylic acid, 22c: mp >260 °C (light brown coloring at 210 °C); IR (KBr) 2400–3600 cm^{-1} (OH and NH_2); ^1H NMR (360 MHz) (DCl–D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.82 (3 H, t, *J* = 7.3 Hz, Me), 0.99–1.07 (2 H, m, CH_2), 1.20 (1 H, d, J_{ab} = 6.7 Hz, C*H*-ring), 1.23–1.49 (4 H, m, 2 C*H*$_2$), 1.52 (1 H, d, J_{ab} = 6.7 Hz, C*H*-ring), 1.58–1.77 (2 H, m, CH_2); ^{13}C NMR (DCl–D_2O; ref 1,4-dioxane = δ 67.4 ppm) δ 14.18 and 14.38 (2 q, (*Me*CH_2CH_2)$_2$, 19.76 and 19.97 (2 t, ($CH_3CH_2CH_2$)$_2$), 25.43 (t, CH_2-ring), 31.56 and 32.56 (2 t, ($CH_3CH_2CH_2$)$_2$), 35.03 (s, Pr_2C), 43.31 (s, *C*-NH_2), 172.67 (s, *C*OOH). Anal. Found: N, 7.41. Calcd: N, 7.56.

2-Amino-4-hydroxy-4-methylpentanoic acid, 23a: mp 253 °C (light sublimation from 236 °C); IR (KBr) 3500–2500 (NH_2, OH, and COOH), 1590–1640 cm^{-1} (CO); ^1H NMR (DCl + D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 1.11 (6 H, s, Me_2), 1.6-2.3 (2 H, m, CH_2), 4.01 (1 H, dd, *J* = 8.4 Hz, *J* = 4.6 Hz, C*H*N); ^{13}C NMR (DCl + D_2O, ref 1,4-dioxane = δ 67.40 ppm) δ 27.62 and 30.73 (2 q, Me), 42.39 (t, CH_2) 52.50 (d, *C*H), 71.45 (s, *C*OH), 174.49 (s, COOH). Anal. Found: N, 9.63. Calcd: N, 9.51.

2-Amino-4-ethyl-4-hydroxyhexanoic acid, 23b: mp 185 °C (light sublimation at 160 °C); IR (KBr) 3600–2500 (NH_2, OH, and COOH) 1560–1630 cm^{-1} (C=O); ^1H NMR (D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.74 and 0.75 (2 × 3 H, 2 t, each *J* = 7.5 Hz, 2 CH_3), 1.3–1.6 (4 H, m, 2 C*H*$_2$Me), 1.68 (1 H, dd, J_{ax} = 10.5 Hz and J_{ab} = 15.5 Hz, H_a, *ABX*), 2.05 (1 H, dd, J_{bx} = 3.1 Hz and J_{ab} = 15.5 Hz, H_b, *ABX*), 3.77 (1 H, dd, J_{ax} = 10.5 Hz and J_{bx} = 3.1 Hz, H_2, *ABX*); ^{13}C NMR (D_2O; ref 1,4-dioxane = δ 67.40 ppm) δ 7.46 and 8.31 (2 q, 2 Me), 29.04 and 31.85 (2 t, 2 *C*H_2Me), 38.68 (t, *C*H_2), 52.88 (d, *C*H), 76.61 (s, *C*OH), 175.86 (s, *C*OOH). Anal. Found: N, 8.10. Calcd: N, 7.99.

3-Amino-5,5-dimethyltetrahydro-2-furanone, 24a (hydrochloride): mp >260 °C (some brown coloring at 260 °C); IR (KBr) 3600–3000 (NH_3^+), 1773 cm^{-1} (C=O); ^1H NMR (DCl + D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 1.73 and 1.46 (2 × 3 H, 2 s, Me_2), 2.18 (1 H, dd, J_{ax} = 12 Hz and J_{ab} = 12.5 Hz, H_a), 2.68 (1 H, dd, J_{bx} = 9 Hz and J_{ab} = 12.5 Hz, H_b), 4.57 (1 H, dd, J_{ax} = 12 Hz and J_{bx} = 9 Hz, H_x); ^{13}C NMR (DCl + D_2O; ref 1,4-dioxane = δ 67.40 ppm) δ 26.71 and 28.58 (2 q, 2 Me), 39.13 (t, CH_2), 50.62 (d, *C*H), 87.13 (s, *C*Me_2), 174.06 (s, C=O).

3-Amino-5,5-diethyltetrahydro-2-furanone, 24b (hydrochloride): mp >260 °C; IR (KBr) 3600–2500 (NH_3^+), 1765 cm^{-1} (C=O); ^1H NMR (DCl + D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.77 and 0.89 (2 × 3 H, 2 t, overlap, *J* = 7 Hz, 2 Me), 1.1–2 (2 × 2 H, m, 2 CH_2), 2.10 (1 H, dd, J_{ax} = 11.5 Hz and J_{ab} = 13 Hz,

H_a), 2.62 (1 H, dd, J_{bx} = 9.5 Hz and J_{ab} = 13 Hz, H_b), 4.51 (1 H, dd, J_{ax} = 11.5 Hz and J_{bx} = 9.5 Hz, H_x); ^{13}C NMR (DCl + D_2O; ref 1,4-dioxane = δ 67.40 ppm) δ 7.71 and 8.14 (2 q, 2 Me), 31.03 and 31.20 (2 t, 2 CH_2), 35.16 (t, CH_2CH), 50.29 (d, CH), 92.40 (s, CEt_2), 174.19 (s, C=O).

3-Amino-5,5-di-*n*-propyltetrahydro-2-furanone, 24c (hydrochloride): mp >260 °C; IR (KBr) 3600-2500 (NH_3^+), 1770 cm^{-1} (C=O); ^1H NMR (DCl + D_2O; ref 1,4-dioxane = δ 3.64 ppm) δ 0.82 (2 × 3 H, t, overlap, 2 Me), 1.0-2.0 (4 × 2 H, m, 4 CH_2), 2.18 (1 H, dd, J_{ax} = 11.5 Hz and 13 Hz, H_a), 2.68 (1 H, dd, J_{bx} = 9 Hz and J_{ab} = 13 Hz, H_b), 4.56 (1 H, dd, J_{ax} = 11.5 Hz and J_{bx} = 9 Hz, H_x); ^{13}C NMR (DCl + D_2O; ref 1,4-dioxane = δ 67.40 ppm) δ 14.39 (q, 2 Me, overlap), 16.98 and 17.38 (2 t, $(CH_3CH_2CH_2)_2$), 36.18 (t, CH_2CH), 39.64 and 40.96 (2 t, $(CH_3CH_2CH_2)_2$), 50.28 (d, CH), 91.79 (s, CPr_2), 174.16 (s, C=O).

Retention Values. Retention values for TLC using Kieselgel 60 F_{254} plates (5 × 10 cm; thickness 0.25 mm) with 1-butanol-acetic acid-water (4:1:1) as eluent: Compound **22a**, 0.43; compound **22b**, 0.60; compound **22c**, 0.65 (ninhydrin positive spots of compounds **22** appeared light purple); compound **23a**, 0.28; compound **23b**, 0.50 (ninhydrin positive spots of compounds **23** appeared dark yellow to light brown); compound **24a**, 0.39; compound **24b**, 0.57; compound **24c**, 0.59 (ninhydrin positive spots of compounds **24** appeared yellow).

Registry No. **11a**, 4091-39-8; **11b**, 13280-00-7; **11c**, 2832-55-5; **11d**, 2907-70-2; **12a**, 78827-37-9; **12b**, 118231-23-5; **12c**, 118231-24-6; **12d**, 128753-38-8; **14a**, 118231-19-9; **14b**, 118231-20-2; **14c**, 118231-21-3; **14e**, 128753-39-9; **14f**, 128753-40-2; **21a**, 123445-50-1; **21b**, 123445-51-2; **21c**, 123445-52-3; *cis*-**18f**, 128753-41-3; *trans*-**18f**, 128753-42-4; **19a**, 114809-78-8; **22a**, 123445-53-4; **22b**, 123445-54-5; **22c**, 123445-55-6; **23a**, 104302-37-6; **23b**, 123445-56-7; **24a**·HCl, 15722-67-5; **24b**·HCl, 128753-43-5; **24c**·HCl, 128779-80-6; **25a**, 128753-44-6; **25b**, 128753-45-7; **26a**, 128753-46-8; **26b**, 128753-47-9; **27a**, 128753-48-0; **27b**, 128753-49-1; **28a**, 128753-50-4; **28b**, 128779-81-7; **29a**, 128753-51-5; **29b**, 128779-82-8.

Syn-Anti Conformational Analysis of Regular and Modified Nucleosides by 1D ^1H NOE Difference Spectroscopy: A Simple Graphical Method Based on Conformationally Rigid Molecules

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The 1D ^1H NOE difference spectra of 50 base- and/or sugar-modified nucleosides were measured in $(CD_3)_2SO$ with irradiation of various protons. The resulting NOE data were used for a conformational analysis with respect to their syn-anti conformer equilibrium. Irradiation of H-8 [purine numbering (purine and pyrimidine numbering has been used for all nucleosides throughout the paper)] of the conformationally fixed compounds **25**β and **41**β resulted in NOE values at H-1′, H_β-2′, and H_β-3′, respectively, which were used to establish a calibration graph for a semiquantitative estimation of syn and anti conformer populations of β-D nucleosides. Moreover, the preferred conformations of a number of α-D nucleosides were qualitatively deduced from their 1D ^1H NOE difference spectra. Measurement of the NOE spectra of sterically hindered nucleosides implied that both the chemical properties as well as the van der Waals radius of a nucleobase substituent are of decisive importance for the conformation around the N-glycosylic bond.

Introduction

Nucleosides exist in solution in distinct conformational ranges with a Gaussian distribution of conformer populations within these ranges. Concerning the overall shape of a nucleoside, the most important conformational parameters are the torsion angle around the glycosylic bond (χ) as well as the sugar puckering, which are interdependent.[1] The energy barrier between the different conformational states is usually low (≈25 kJ/mol),[2,3] implying dynamic equilibria between them which might be more or less biased toward one side. Normally, the different conformational modes can be reliably described by two-state models (syn-anti; N/S-type sugar puckering).[1]

Rare nucleotides characterized by unusual aglycon moieties like 2-thiouridine, pseudouridine, wyosine (**42**β), and others are particularly common in tRNA's. Some of these manifest either the syn or the anti conformation around the glycosylic bond in the solid state.[4] Moreover, the syn-anti equilibrium is a key event in going from a right-handed B-DNA to a left-handed Z-DNA involving a conformational change in guanylate residues from anti to syn.[5,6]

As substrate or inhibitor activities of nucleosides and nucleotides might be correlated with their preferred structure in solution, the evaluation of thermodynamic and conformational parameters is of key importance in eluci-

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