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Approaches to Analogs of Anhydrogliotoxin

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Abstract: The addition of α-halo-α-aminoacyl chlorides to ethyl indolenine-2-carboxylates followed by reaction with sulfur nucleophiles and a final ring closure provides a convenient and new synthetic scheme to analogs of gliotoxin, the simplest of the natural products containing the epidithiodiketopiperazine system. Illustrative of this approach, adducts of ethyl 3,3-dimethylindolenine-2-carboxylate (17) with acid chlorides, α-halo acid chlorides, and N-trifluoroacetyl-α,α-dichloroacryloyl chloride (44) have been studied. The last adduct when treated with a sulfide—polysulfide mixture gave a monosulfide 49 (30% yield) but no disulfide 50. Reduction of 49 with NaCNBH3 proceeded stereoselectively to afford mainly the secogliotoxin analog 51 in addition to the diastereoisomer 52. Cyclization of this mixture presumably led to the strained epimonothiodiketopiperazine 41, which easily opened to the isomeric lactam 55 in addition to lactam 56 formed by epimerization.

The number of natural products containing the epidithiodiketopiperazine ring continues to grow with the recent reports on the two fungal metabolites chaetocin (2) and verticillin A (3). Both are highly active against gram-positive bacteria and viruses while the latter has only antifungal and antiviral activity.

The mechanism of antiviral action of gliotoxin and aranotin depends upon the specific inhibition of RNA-dependent DNA polymerases from tumor-producing viruses or blocking of the synthesis of viral RNA in the case of chetomin.

Several syntheses of simple epidithiodiketopiperazines have been reported, which feature the addition of sulfur substituents to a preformed diketopiperazine. Surprisingly, the simple model 1a is highly active in inhibiting viral RNA synthesis, in support of the view that the activity of the more complex natural products resides in the epidithiodiketopiperazine ring.

Another approach to this ring system started with 2-benzamido-2-mercaptopropanoic acid (13) as a possible precursor.

The drastic reaction conditions of all of these methods preclude their successful extension to the polycyclic epidithiodiketopiperazines. A synthetic approach of general applicability, we felt, would feature the initial construction of the disulfide bridge and then ring closure to a bridged diketopiperazine.

The addition of acyl chlorides to indolenines (Chart I), a reaction first reported by Leuchs, who studied compounds 14-16, served as our first step.

The 2-chloro substituent in Leuchs’ adducts 18-20 is known to undergo easy nucleophilic displacement, and...
The azo ester 23, when prepared from ethyl \( \alpha \)-isopropylacetooacetate and benzenediazonium chloride under mildly alkaline conditions, was stable enough to permit isolation. Careful treatment with ethanolic solutions of sodium hydroxide or preferably ammonium hydroxide gave the hydrazone ester 24, which was converted into 17 by refluxing in HCl-saturated ethanol. The yields are much higher than reported in the published procedure\(^{31}\) where the coupling and hydrazone

formation steps are carried out under such strongly alkaline conditions that only the hydrazone 25 can be isolated. This, on Fischer cyclization, gives a mixture of 17 and 26 accompanied by 2,3-dimethylindole, the product of decarboxylation and rearrangement of 26. The indolenine 17 can also be prepared by refluxing 23 in absolute alcoholic hydrogen chloride. This indicates that the transformation (23 \rightarrow 24) in the Japp-Klingemann reaction can also be acid catalyzed.

We first examined the reaction of 17 with simple acid chlorides, such as acetyl chloride and chloroacetyl chloride, and found that when freshly purified reagents were employed, the Leuchs addition proceeded in high yield at room temperature. Interestingly, this is the first instance of addition of acyl chlorides to an indolenine-2-carboxylic acid derivative, the previous examples being limited to indolenine with 2-hydrogen, 2-methyl, or 2-phenyl substituents. The indolenine 17 is less reactive than unconjugated ones, since benzoyl chloride could not be added. Reaction of 17 with ethoxycarbonyl chloride or benzyloxycarbonyl chloride was very slow, and trifluoroacetyl chloride did not react at all.

Two isomeric thioacetates, 28 and 29, were isolated when potassium thioacetate was allowed to react with the product from acetyl chloride and 17 which had been allowed to warm to 40°, presumably as the result of a Plancher rearrangement (21 \rightarrow 27, Chart III). With potassium thiocyanate on 21, the 2-isothiocyanato compound 30 (Chart IV) was isolated instead of the expected 2-thiocyanato compound. Therefore, we expected the 2-isothioureido derivative 34, but, under these conditions, isolated starting material 17.

The pKₐ of 33 was measured and found to be 7.7. When the solvolysis of 33 was attempted at pH 9.5 in the hope that 34 might be more stable as a neutral species, still only 17 was isolated. This suggests that unconjugated indoline-2-thiols are inherently unstable. Likewise, 2-indolinols are known only as N-acyl or N-alkyl derivatives. At least these reactions prove that no Plancher rearrangement occurs at room temperature during acyl chloride additions or subsequent displacement reactions.

When 32 reacted with inorganic sulfides, such as ammonium sulfide, sodium mono-, di-, or tetrasulfide, or thiocarbonate, two products resulted: a mono- (37)

and a disulfide (38) in yields varying with the reactant (Chart VI). Sodium sulfide and sodium thiocarbonate

gave mainly the monosulfide 37 (ca. 40\% yield), whereas ammonium sulfide and sodium di- and tetrasulfide, 
which all exist as mixtures of mono- and polysulfides, gave the mono- and disulfide in proportions of 2:1, 1:4, and 2:7, respectively.

None of the thiol 36 could be detected; this together with the observation that sodium sulfide gives mainly (>90\%) the monosulfide 37 suggests that 37 as well as 38 arise from an intramolecular displacement of chlorine in the sulfhydryl intermediate 35 (n = 1 or 2) and not via the dithiol 36. A tetrasulfide 39 could not be detected although such a ring system forms easily in thio-bridged diketopiperazines.23

Models indicate that a cyclic sulfide is possible only in structures 37 and 38. For the disulfide, but not the monosulfide, an alternate structure 40 may be envisaged. The disulfide 38, however, could be converted quantitatively into the monosulfide 37 with triphenylphosphine37 as evidence that no rearrangement occurred in the formation of the disulfide. The nmr spectra of 37 and 38 show a surprisingly large difference in the \( \delta \) value for the aromatic C\(_7\) proton (\( \delta \) 7.70 and 8.20, respectively), indicative of increased deshielding by the carbonyl group in 38.

An N-acylated 9-amino analog of 38 on deacylation might undergo spontaneous ring closure and formation of the dithio-bridged diketopiperazine 41 (n = 2), an analog of dehydrogliotoxin (12b).

Accordingly, N-trifluoroacetylserosine chloride (42) was prepared from the free acid with thionyl chloride,39 conditions mild enough not to affect the trifluoroacetyl group.40 When 42 was refluxed in sulfuryl chloride in an attempt to prepare 43, the \( \alpha \)-dichloro acid chloride 44 was isolated. Details on this synthesis as well as some reactions of this interesting compound have been reported elsewhere.41

When the addition product from 44 and 17 was allowed to react with sodium tetrasulfide, a ninhydrin-positive, crystalline compound was isolated in 30\% yield whose structure agrees with 49 (Chart VII).

As we have proposed elsewhere,44 44 may decompose spontaneously to form 47, which may then react with 17 to give 48 which in turn forms 49 with polysulfide ions in an intramolecular reaction (pathway A). Alternatively, pathway B proceeds via 45, the addition product of 44 and 17, which may then react in either or both of two ways: base-catalyzed hydrolysis of the N-trifluoroacetyl group to yield 49 via 48 (pathway B\(_1\)) or removal of the N-trifluoroacetyl group following reaction with polysulfide ions (pathway B\(_2\)). At the moment, we lack the definitive evidence necessary for a decision among these mechanistic possibilities.

We were unable to detect the disulfide 50, possibly because it is either inherently unstable, or unable to survive the strongly alkaline conditions of the tetrasulfide reaction.42

The monosulfide 49 was reduced with sodium cyanoborohydride44 to the amines 51 and 52 (Chart VIII), which are secogliotoxin analogs.

The course of the reduction is guided by steric induction of the carboxethoxy group. The nmr spectrum of the reduction mixture showed two signals for the C\(_8\) proton, at \( \delta \) 5.65 and 5.29 in the ratio 2:1, respectively, and two signals at \( \delta \) 2.54 with a separation of 2 Hz for the N-methyl group. It is assumed that the C\(_8\) proton in the stereoisomer 52 is more shielded than in 51, so that the signals at \( \delta \) 5.65 and 5.29 can be assigned to structures 51 and 52, respectively, of which 51 is the major (66\%) and the diastereomer 52 the minor product (33\%). An

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(37) This method has been used to convert dehydrogliotoxin (12b)43 and sporidesmin (44)44 into monosulfides.


(42) The alkaline decomposition of organic disulfides very often produces monosulfides.43


epithiodiketopiperazine can be formed only from 51 in which ester and amine functions are in a cis relationship. A bulkier hydride donor might make the reduction even more stereoselective.

Surprisingly the conversion of 49 into 51 and 52 led to no change in the ir spectrum of the amide carbonyl absorption (1705 cm\(^{-1}\)). This suggests that conjugation in O—CC=NCH\(_3\) has little effect.

The mixture of monosulfides 51 and 52 was heated with ethanol in an attempt to form the epimonothiodiketopiperazine 41 (n = 1). Only in a sealed tube at 125\(^\circ\) did a reaction occur yielding, besides starting material, a compound with a slightly higher R\(_f\) value on tlc. This compound had nearly the same mass spectrum as the starting mixture, with differences only in peak intensities, indicative of closely related isomers of 51 and/or 52. The nmr spectrum could best be interpreted as a mixture of structures 55 and 56 (Chart IX).

The occurrence of 55 and 56 would also be explained by epimerization at C\(_{2'}\) in 55. A deuterium-exchange study is planned to check this possibility.

The occurrence of two pairs of enantiomers could then be explained by epimerization at C\(_{2'}\) in 55. A deuterium-exchange study is planned to check this possibility.

Particular attention was given to these considerations, for if only one pair of enantiomers had been formed, this probably would have been 55, derived only from the reactive starting material 51, via the desired diketopiperazine 41 (pathway A, Chart IX). A Dreiding model shows that the epithiodiketopiperazine ring system in 41 is a highly strained though not an impossible one as has been shown by Taylor.

The occurrence of two pairs of enantiomers could then be explained by epimerization at C\(_{2'}\) in 55. A deuterium-exchange study is planned to check this possibility.

The occurrence of 55 and 56 would also be explained by pathway B, Chart IX. If the amide groups in 51 and 52 were cleaved by ethanol, the \(\alpha\)-thio-bridged \(\alpha\)-amino acid esters 53 and 54 would result. These could lactamize in two ways, yielding besides the starting materials the structures 55 and 56, respectively. Structures 53 and 54 with an unacylated \(\alpha\)-thio amino acid moiety are undoubtedly unstable (see also Chart V and accompanying text), and should break down to the indolenine ester 17. However, the reaction mixture 51 + 52 \(\rightleftharpoons\) 55 + 56 showed only two spots on tlc with no trace of side products, making this mechanism unlikely.

Milder reaction temperatures and the use of nonprotic solvents provided no new information. At 90\(^\circ\) the formation of the new isomers is very slow and no new component could be detected; diglyme as solvent at 90 or 120\(^\circ\) failed to give any identifiable product. At present there is no evidence permitting a choice between pathways A and B.

**Experimental Section**

Infrared spectra were measured with Perkin-Elmer spectrophotometers, Models 237B (CHCl\(_3\) or CCl\(_4\)) and 421 (KBr), and uv spectra with a Cary Model 11 (95% EtOH). Mass spectra were obtained with the double-focusing Hitachi RMU-6E mass spectrometer. Proton magnetic resonance spectra were measured on the Varian Associates Model A-60 spectrometer. Chemical shifts are reported as \(\delta\) values (ppm) relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated.

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(45) Under these conditions compound 37 was found to be stable.

(46) Poyer and Rae\(^{27}\) described the synthesis of 13 and 2,2'-dibenzamidino-2,2'-dithiodipropanoic acid, in which the amino function is acylated. Interestingly, the decylated products were not mentioned.

(47) A Plancher rearrangement\(^{27}\) producing 57 and 58 cannot be completely ruled out. The shift in amide carbonyl absorption from 1690 to 1690 cm\(^{-1}\) which accompanies this reaction is somewhat unexpected. Although the latter absorption is normal for a tertiary amide, it could also indicate that the amide is part of a six-membered ring. Arguing against this possibility, however, is the similarity of the 3-methyl signals in the nmr spectra of starting materials and products.
with ether. The organic layer was washed with water, 5% NaOH at —15°, an oil separated from the dark red colored reaction solution B was added with swirling to solution A and cooled in a dry ice–acetone bath. After the addition of solution B to A, 232.5 ml of 5 M NaOH (1.15 mol), yielded a dark red residue. From this oil, 16.7 g (0.098 mol) of unreacted 22 could be isolated by vacuum sublimation at 90° (1.0 mm). The addition product was purified by vacuum sublimation at 90° (0.75 mm), followed by crystallization from hexane and washing with petroleum ether, to give thin needles, mp 100.5–101.5°; mass spectrum (175°), 731 (M+), 434 (M+ - 2 CO), 191 (M+ - 2 CO), 165 (M+ - 3 CO), 140 (M+ - 4 CO), 319 (M+ - 5 CO), 145 (160 - CO), 105, 92 (alkene - 1).

Ethyl α-Keto-β-methylbutyrate Phenylhydrazone (24). To a solution of 48.0 g (0.175 mol) of the azo ester 23 in 250 ml of ethanol was added 50 ml of concentrated aqueous NH₄OH. The mixture was stirred and kept at 50° while the reaction was monitored by uv spectroscopy (shift from λmax 277 to 325 nm). The reaction was stopped after the starting material had disappeared (45 min). The oil was then separated from the water layer until neutral and dried (Na₂CO₃), and the ether was removed to yield a dark red solid. From this oil, 16.7 g (0.098 mol) of unreacted 22 could be isolated by vacuum sublimation at 90° (1.0 mm). The reaction mixture was then cooled to —15°, was added at once.

Solution B. A solution of potassium azidochloride was prepared from 22 (200 mg, 0.30 mol) of freshly distilled aniline, 255 ml (1.02 mol) of 4 N HCl, and 210 g (0.30 mol) of sodium nitrite dissolved in 50 ml of water. All solutions were cooled at —15°. Immediately after the addition of the sodium nitrite solution, the larger volume was added to the smaller volume with swirling, and the solution was stirred for 45 min at 0°. All solutions were cooled at —15°. The reaction mixture was then cooled to — 15°, was added at once.

Solution A. A solution of 51.0 g (0.30 mol) of ethyl isopropylacetoacetate (22) in 50 ml of concentrated aqueous NH₄OH. The resulting solution was stirred for 1 h 15 min, then the solutions were added to the stirring solution and the reaction mixture was stirred for 2 h; the alcohol was removed and the residue extracted with ether. The ether layer was washed with 5% NaOH and water until neutral and dried (Na₂CO₃), and the ether was removed to yield 545 mg of a yellow oil: uv max 277 nm (shoulders at 215 and 330).

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Solution B. A solution of potassium azidochloride was prepared from 22 (200 mg, 0.30 mol) of freshly distilled aniline, 255 ml (1.02 mol) of 4 N HCl, and 210 g (0.30 mol) of sodium nitrite dissolved in 50 ml of water. All solutions were cooled at —15°. Immediately after the addition of the sodium nitrite solution, the larger volume was added to the smaller volume with swirling, and the solution was stirred for 45 min at 0°. All solutions were cooled at —15°. The reaction mixture was then cooled to —15°, was added at once.

Solution A. A solution of 51.0 g (0.30 mol) of ethyl isopropylacetoacetate (22) in 200 ml of ethanol was cooled at —15° (ice–salt bath). Just before the addition of solution B to A, 232.5 ml of 5 M NaOH (1.15 mol), was added at once.

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crystallization from ethanol-hexane for 37 (mp 103.5-104.5°) and justed at 5.2 or in another experiment to 9.5 with 0.5 cm⁻¹; nmr

The product with lower

2

4.03 (d, 1 H, C

3.70 (d, 1 H, Q-Hp), 1.45 (s, 3 H, C

with 3 % ethanol-benzene and one time with 2 % ethanol-benzene). In the other, the disulfide

1475, 1455, 1390, 1368, 1284, 1130, 1100, and 1025 cm⁻¹; nmr

2

3.86 (s, 3 H, NCH₃), 1.51 (s, 3 H, C

value was found to be the monosulfide

19.82. Found: C, 55.93; H, 5.17; N, 4.39; S, 19.50.

Disulfide 38 into Monosulfide 37. A solution of 13 mg (4.1 x 10⁻² mmol) of 38 and 22 mg (8.4 x 10⁻³ mmol) of triphenylphosphine in 1 ml of absolute ethanol was kept, wrapped in aluminum foil, at room temperature for 20 days. The reaction was monitored by tic. The solvent was removed and the residuesubject to preparative tic (developed two times, 3 % ethanol-benzene), to yield 9.5 mg (3.25 x 10⁻⁴ mmol, 80%) of 37. Identical

C₇H₇N₄O₄S₂; m/e

28 mg (59 % and 60 %, respectively) of crystalline material having Rf values, uv, and ir spectra identical with those of 17.

2-Carbethoxy-3,3-dimethylindolinol2,1-b-thiazolidinone-8 (37 and 38).⁵⁰ The addition product 32 of 1.085 g (5 mmol) of 17 and 6.0 g diglyme was divided into five 5-ml aliquots. To the first sample was added quickly with ice cooling 20 ml of a freshly prepared, ice-cold solution of sodium disulfide (1.1 M) in water. The latter was prepared the following way: 3.4 g (0.11 mol) of powdered sulfur was dissolved in a solution of 26 g (0.11 mol) of Na₂S·9H₂O in ca. 70 ml of water by stirring for 15 min, after which the clear solution was then diluted to 100 ml. To minimize saponification, the reaction time was kept less than 5 min. The reaction was stopped by acidification with 2 N HCl. The mixture was extracted with ethyl acetate, and the organic layer was filtered (to remove S₈), washed with water until neutral, and dried (Na₂SO₄). The solvents were removed under high vacuum to yield 313 mg of a yellow oil, which showed on tic (4 % ethanol-benzene) two spots with Rf values of 0.48 and 0.55, respectively. Two fractions (24 mg, 8 %, and 103 mg, 32 %) could be isolated by preparative tic (developed one time with 3 % ethanol-benzene and one time with 2 % ethanol-benzene). The product with lower Rf value was found to be the monosulfide 37; the other was the disulfide 38. Both compounds could be further purified by vacuum sublimation (120° (0.7 mm)) followed by recrystallization from ethanol-hexane for 37 (mp 103.5-104.5°) and from ethanol-water for 38 (mp 111-113°).

Monosulfide 37: ir (CHCl₃) 2960, 1730 (ester), 1705 (amide), 1645 (methylimino), 1475, 1455, 1390, and 1290 cm⁻¹; nmr

1.20 (t, 3 H, CH₃), 2.13 (t, 3 H, C₉-H₉); mass spectrum (180°), m/e 283 (M⁺), 267, 251, 235, 221, 205, 191, 177, 163, 150, 138, 126, 114, 102, 90, 78, 66, 54, 42, 30, 18, 16, 14, 12, 10, and 8.

Anal. Caled for C₂H₇N₄O₄S₂: C, 61.83; H, 5.88; N, 4.81; S, 13.58. Found: C, 62.47; H, 5.82; N, 4.54; S, 13.07.

Disulfide 38: ir (CHCl₃) 2980, 1732 (ester), 1650 (amide), 1595, 1460, 1390, and 1290 cm⁻¹, nmr

N'-Trifluoroacetyl-N,N-dichlorosarcosyl Chloride (44). The synthesis of this compound (bp 54-58° (40 mm)) is described elsewhere.⁴¹

2-Carbethoxy-3,3-dimethyl-9-methylnindolinol2,1-b-thiazolidinone-8 (49). The addition product 1.52 g (7 mmol) of 17 and 3.8 g (14 mmol) of 44 in 25 ml of dry benzene was prepared as described for the preparation of 38 (stirred 15 min) and yielded 3.86 g of a light yellow oil. To an ice-cold solution of this oil in 30 ml of dry diglyme was added quickly an ice-cold, freshly prepared solution of sodium tetrasulfide. The latter was prepared analogous to the preparation of Na₂S from 10.2 g (33.0 mol) of powdered sulfur and 26 g (0.11 mol) of Na₂S·9H₂O. Reaction conditions and work-up were the same as described above. After preparative tic, 42 mg (14 %) of 37 and 154 mg (47 %) of 38 could be isolated besides 26 mg (12 %) of the starting material 17.

Conversion of Disulfide 38 into Monosulfide 37. A solution of 13 mg (4.1 x 10⁻² mmol) of 38 and 22 mg (8.4 x 10⁻³ mmol) of triphenylphosphine in 1 ml of absolute ethanol was kept, wrapped in aluminum foil, at room temperature for 20 days. This was prepared analogous to the preparation of Na₂S from 10.2 g (33.0 mol) of powdered sulfur and 26 g (0.11 mol) of Na₂S·9H₂O. Reaction conditions and work-up were the same as described above. After preparative tic, 80 mg (27 %) of monosulfide 37 and 40 mg (12.4 %) of disulfide 38 were isolated.

Two other 5-maliquots were allowed to react with an aqueous solution of sodium monosulfide (20 ml of a 1.1 M solution), or freshly prepared sodium thioacetate⁵¹ (20 ml of a 1.1 M solution), respectively. Reaction conditions and work-up were as described above. The solution was dried, neutralized to pH 4 with 2 N HCl and extracted three times with 4 % ethanol-benzene) to yield 192 mg (0.88 mmol, 38 %) of 37. The reaction mixture was monitored by tic.

Conversion of Disulfide 38 into Monosulfide 37. A solution of 13 mg (4.1 x 10⁻² mmol) of 38 and 22 mg (8.4 x 10⁻³ mmol) of triphenylphosphine in 1 ml of absolute ethanol was kept, wrapped in aluminum foil, at room temperature for 20 days. The reaction was monitored by tic. The solvent was removed and the residue subjected to preparative tic (developed two times, 3 % ethanol-benzene), to yield 9.5 mg (3.25 x 10⁻⁴ mmol, 80 %) of 37. Identical

C₇H₇N₄O₄S₂; m/e

258 (M⁺), 236, 218, 201, 193, 185, 170, 152, 134, 116, 98, 80, 62, 44, 26, 18, 16, 14, 12, 10, and 8.


One 5-maliquot was treated with 20 ml of an aqueous ammonium sulfide solution (7 %). Reaction conditions and work-up were the same as described above. After preparative tic, 80 mg (27 %) of monosulfide 37 and 40 mg (12.4 %) of disulfide 38 were isolated.

(50) According to IUPAC rules the nomenclature for 37 and 38 should be 2,3,9,9a-tetrahydro-3-keto-9,9-dimethyl-9a-carboxethoxathi­zolo[3,2-α]-indole and 3,4,5,6,10a-hexahydro-4-keto-10,10-dimethyl­10a-carbethoxy-1,2,5,8-dihiazin[5,6-d]indole, respectively. For convenience we use the names above.

mmol) of the mixture of 51 and 52 in 5 ml of absolute ethanol was heated in a sealed ampoule at 108° for 24 hr and then at 125° for 16 hr. Tlc (6% ethanol–benzene) showed the presence of only two products, the starting material and a product with larger Rf. The solvent was removed and the brown oily residue subjected to preparative tlc (developed three times with 5% ethanol–benzene), to yield 26 mg (65%) of "starting material" and 14 mg (35%) of isomerized product: tlc (6% ethanol–benzene) only one spot, Rf 0.50; ir (CHCl3) 3400 (sharp, NH), 2980, 2940, 2860, 1730 (ester), 1640 (amide), 1600, 1525, 1480, 1395, and 1370 cm⁻¹; nmr δ 8.15 (mult, 1 H, Cr-H), 7.20 (mult, 3 H, C4-b-H), 5.50 (broad singlet, 1 H, Q'-H), 4.17 (q, 2 H, CH3CH3), 3.23 and 3.13 (2 singlets, separated 5 Hz, 3 H, N-CH3), 2.0 (broad S, 1 H, NH), 1.47 and 1.29 (2 singlets, 6 H, CH3CH3), 1.29 (t, 3 H, CH3CH3). Mass spectrum (160°), m/e 320 (M+), identical with that for 51 and 52, except for a stronger signal at m/e 304 (M+ − CH4) and a weaker one at m/e 247 (M+ − CO2C2H5) and 245.

The nmr spectrum of the isolated "starting material" showed a change in that the ratio of the two signals from the Cg-proton was reversed (now δ 5.65/5.29 = 1:2), indicating that only the cis enantiomers 51 have been isomerized.

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