Synthesis and Absolute Configuration of Wybutine, the Fluorescent Minor Base from Phenylalanine Transfer Ribonucleic Acids

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The phosphonium chloride 6 having an optically active amino acid moiety was synthesized from (S)-serine benzyl ester tosylate (2b) through a six-step route. The utility of 6 as a reagent for the Wittig reaction was exemplified in the olefination with benzaldehyde, affording the (E)- β,γ-unsaturated amino acid derivative 11 as a sole geometrical isomer. This new method of amino acid homologation was successfully employed for the first chiral synthesis of wybutine (1c), the minor base isolated from yeast phenylalanine transfer ribonucleic acids: the Wittig reaction between 6 and the tricyclic aldehyde 16 followed successively by methylation and catalytic reduction afforded 1c. Comparison of wybutine with synthetic 1c has unequivocally established that wybutine has an S configuration.

Keywords wybutine; Wittig reaction; β,γ-unsaturated amino acid; chiral synthesis; amino acid homologation; phenylalanine transfer ribonucleic acid; hypermodified base; stereoselective olefination; alanine synthon; absolute configuration

The base sequences of more than 450 transfer ribonucleic acids (tRNAs) have been determined since the publication of the pioneering work by Holley et al. on yeast alanine tRNA in 1965. The most prominent feature of tRNAs is a frequent occurrence of modification at the base moiety, and more than 50 modified nucleosides or bases from tRNAs have been characterized. RajBhandary et al. determined the base sequence of yeast phenylalanine tRNA (tRNA^Phe) and discovered a fluorescent component at the next position to the 3'-end of the anticodon. The chromophore was associated as the nucleoside, wybutosine whose N-glycosidic bond was shown to be unusually susceptible to acid hydrolysis by Thiebe and Zachau. Thus the fluorescent base, wybutine, was selectively obtained by mild acid treatment of the tRNA, and the structure 1c was assigned to this base by Nakanishi's and Zachau's groups without determining the stereochemistry. One of the fluorescent bases isolated from rat and calf liver tRNAs^Phe was also reported to be wybutine. The congeners 1a, b, d—f have subsequently been isolated from tRNAs^Phe of various eukaryotic species and unfractinated tRNAs of archaeabacteria.

The chemical structures of the members of the family 1 are quite unique because they embody not only a 3-methylguanine skeleton but also a condensed tricycle: no other 3-methylguanine derivative has been reported to occur naturally and no condensed tricyclic base other than 1 has been found in nucleic acids. Nakanishi et al. achieved a synthesis of the racemic modification of 1c by cyclodegradation of 3-methylguanine with (±)-5-bromo-2-[(methoxycarbonylamino)-6-oxoheptanoate, ascertaining the correctness of the two-dimensional structure of wybutine. They also reported that the reaction of 7-benzyl-3-methylguanine with the bromoketone followed by catalytic hydrogenolysis gave a better result. The absolute configuration of wybutine was reported to be S by comparison of the circular dichroism (CD) spectrum of N-(methoxycarbonyl)glutamic acid dimethyl ester, obtained from a degradation product of wybutine, with that of an authentic sample derived from (S)-glutamic acid. Unfortunately, the dimethyl ester obtained from wybutine was not fully characterized and there is an inconsistency in this report. Since wybutine is available in only a minute quantity from tRNA^Phe, we planned a synthesis of optically active 1c for unambiguous determination of the absolute configuration of wybutine. This paper reports the first chiral synthesis of 1c.

In preceding papers, we described some model experiments for construction of a side chain at the 7-position of 1-benzylwyne, and found that the Wittig reaction on 1-benzyl-7-formylywe (16) was most promising. Along this line, the phosphonium reagent 7a was required for the preparation of 18, which appeared to be a good intermediate for the synthesis of not only 1c but also its congeners 1d—f. We first attempted to synthesize 7a (TsO- for I-) by metathesis of the tosylate 4a, which is easily accessible from (S)-serine methyl ester hydrochloride (2a: X = Cl) according to the procedure reported for the N-benzylxyocarbonyl analog. The reaction of 4a with triphenylphosphine in N,N-dimethylformamide (DMF), however, took place only sluggishly at 40 °C and that at a more elevated temperature failed to afford the pure phosphonium salt. We then converted 4a into the iodide 8a by following the general method. The reaction of 8a with triphenylphosphine in DMF at 50 °C gave a mixture, whose proton nuclear magnetic resonance (1H-NMR) spectrum indicated the presence of 7a and methyltriphenylphosphonium iodide. When the reaction was conducted in toluene under reflux, 2-[methoxycarbonyl]amino]-2-propenoic acid methyl ester, a product formed via β-elimination, was also obtained. Although the product obtained in the reaction at 80 °C was still contaminated with methyltriphenylphosphonium iodide, prolonged reaction at 50 °C gave pure 7a in good yield. Nevertheless, treatment of 7a with n-butyllithium in tetrahydrofuran (THF) at -78 °C followed

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by addition of 1-benzyl-7-formylwye (16) resulted in the 
\[ \beta \]-elimination in preference to the Wittig reaction. We 
considered that the corresponding phosphonium salt 6 with 
a free carboxy group might not undergo the \[ \beta \]-elimination in 
view of a successful precedent with (2-carboxyethyl) 
triphenylphosphonium chloride. 19 An attempt to obtain 6 
by treatment of 7a with sodium hydroxide in aqueous 
methanol again resulted in the \[ \beta \]-elimination. We then 
prepared 7b through the iodide 8b, which was synthesized 
from (S)-serine benzyl ester p-toluenesulfonate (2b, 
X = TsO), 20 in a manner similar to that described for 7a. 
Although direct hydrolysis of 7b was not achieved, 
conversion of the iodide 7b into the chloride followed by 
hydrolysis over Pd-C afforded 6 in 59% overall yield 
base on (S)-serine. We failed in an alternative synthesis 
of 6 (1 for Cl) by the reaction of triphenylphosphine with 
(R)-3-iodo-N-(methoxycarbonyl)alanine (9), which was 
obtained by hydrolysis of 4b followed by metathesis 
with sodium iodide, because of its poor reactivity.

In order to examine the behavior of 6 in the Wittig 
reaction, we first performed the reaction of 6 with 
benzaldehyde using 3 molar eq of n-butyllithium in a mixed 
solvent of THF and hexamethylphosphoric triamide 
(HMPA), obtaining [S-(E)]-2-(methoxycarbonyl)amino-4-
phenyl-3-butenoic acid (11) (isolated as the methyl ester 13 
in 28% yield). Even the use of 2 molar eq of the base 
afforded 11, but in somewhat lower yield. An E configuration 
of 11 was assignable on the basis of the coupling constant 
(16 Hz) observed for the olefinic protons. The formation 
of the (Z)-isomer of 11 was not observed by \[ \beta \]-NMR 
spectroscopy, although nonstabilized triphenylphosphorus 
ylides often produce (Z)-alkenes preferentially. 21 The 
observed stereoselectivity may be interpreted by analogy 
with the preponderance of (E)-alkenes reported for similar 
reactions of nonstabilized ylides bearing an oxido, 
carboxylato, or amidoo anion, 21b but nevertheless, such a 
marked preference for the (E)-isomer in the reaction of 6 
is noteworthy. A major side product of this reaction was 
the phosphine oxide 15. On hydrogenation over Pd-C 11 
afforded (S)-2-[(methoxycarbonyl)amino]-4-phenylbutanoic 
acid (12) in 27% overall yield. The specific rotation 
of this sample was identical with that of an authentic 
 specimen of 12 \([\alpha]_{D}^{25} +30.2^\circ \text{(MeOH)}\) derived from 
(S)-homophenylalanine (10). 22 We can not, however, say 
whether the configuration of the chiral center was 
completely retained throughout these transformations, since 
we can not rule out the possibility that 12 thus obtained 
from 6 was contaminated by a trace of 15 having a large 
specific rotation \([\alpha]_{D}^{25} +98.5^\circ \text{(MeOH)}\) because of the 
difficulty of purification. Compound 12 was then converted 
into the methyl ester 14, from which the methyl ester of 15 
was easily removable by chromatography. The specific 
rotation of 14 thus obtained was 92% of that of an 
authentic sample derived from 10 via 12. 23

The next step toward access to wybutine should be
utilization of the aldehyde 16 as a substrate of the present method of constructing optically active \( \beta,\gamma \)-unsaturated amino acid derivatives. However, the reaction between 16 and the phosphorane generated from 6 gave poorer results than the case of benzaldehyde and proved to depend markedly on subtle changes of the reaction conditions. The best result was obtained when 6 was dried over molecular sieves in a mixture of THF and HMPA, then treated with 3 mol eq of \( n \)-butyllithium at \(-78^\circ C\), and the resulting phosphorane was then allowed to react with 16 at \(-78^\circ C\) to \(-18^\circ C\). Flash chromatography\(^{149}\) of the crude product afforded unchanged 16 (28% recovery), a mixture of products, whose \(^1H\)-NMR spectrum suggested that the main component was 19, and a polar fraction containing the desired acid 17. Compound 19 should be a product formed through vinylogous aldol condensation. For obtaining 17, it was indispensable to dry the solution of the phosphonium salt 6 over molecular sieves before use; otherwise no 17 was formed, but 15 was produced in 83% yield. When the temperature of the Wittig reaction was raised to room temperature at the final stage, a small amount of the rearranged product 20a was suggested to be formed. Although we obtained its methyl ester 20b as a mixture with the methyl ester of 15, we could not purify it because of the instability of this type of compounds.\(^{149}\) Compound 20b\(^{23}\) was also obtained as a yellow oil in 3% yield by the Wittig reaction using sodium (methylthiolsulfinyl)methanide in dimethyl sulfoxide at room temperature followed by methylation. We previously reported on this type of rearrangement of a model compound under similar conditions.\(^{149}\) Analogous transformations through cleavage of a pyrimidine ring followed by recyclization have been recorded.\(^{26}\)

Compound 17, which was contaminated with other polar products, was isolated as the methyl ester 18 by flash chromatography after treatment with trimethylsilyldiazomethane\(^{27}\) in 16% yield.\(^{28}\) In this case again, no (Z)-isomer of 18 was detected by \(^1H\)-NMR spectroscopy. It should be noted that the \( \beta,\gamma \)-unsaturated amino acid ester 18 underwent racemization when the methylated mixture was allowed to stand at room temperature without aciddization. This was probably caused by contaminating bases such as 21.\(^{29}\) Indeed, 16, which should be formed through cyclization of 21, was detected by means of \(^1H\)-NMR spectroscopy in the methylated mixture. The \(^1H\)-NMR spectrum of 18 is in accord with that of a model compound, (E)-1-benzyl-7-(3-methyl-1-butenyl)wye (22),\(^{149}\) except for the C(γ)-olefinic proton, which is more deshielded by 0.5 ppm than the corresponding one [C(1')-H] of 22. The ultraviolet (UV) spectrum of 18 [\( \lambda_{\text{max}}^{\text{BOD}} \) 254 nm (ε 25500), 282 (sh) (8200), 324 (5200)].\(^{149}\) This should stem from the different extent in conjugation of the exocyclic double bond with the tricycle, because the UV spectrum of compound (±)-23\(^{20}\) with a saturated side chain was practically identical with the spectra of the model compounds 24.\(^{149}\) A consideration of a space-filling molecular model for 22 suggested that the exocyclic double bond should be arranged as the s-cis form so that the maximum conjugation is attained. This was supported by the nuclear Overhauser effect (NOE)\(^{31}\), when the C(6)-Me resonance was irradiated, the enhancement obtained for C(2')-H was 22%, whereas that for C(1')-H was only 3%. With 18 also, saturation of the C(6)-Me resonance gave 28% and 2% enhancements of the intensities of the C(β)-H and C(γ)-H signals, respectively, indicating that there was no substantial difference in conformation between 22 and 18. It is unlikely that the inductive effect of the functional groups of 18 alone brings about the UV spectral difference. We might suppose that the exocyclic double bond in 18 is forced slightly out of the plane of the heterocycle owing to an intramolecular hydrogen bonding between the amino hydrogen at the side chain and the carbonyl oxygen at the 9-position to cause some decrease in the conjugation. However, we found no evidence for such hydrogen bonding in the stretching absorption band due to the carbonyl at the 9-position. A π-stacking interaction between the phenyl
of the 1-benzyl group and the carbamate of the side chain might be another factor in the supposed deviation of the coplanarity. In such a defined conformer, the C(γ)-H resonance observed at unexpectedly low magnetic field may be interpreted as a result of the anisotropic effect of the carbamate group.\(^{22}\)

Now that a key intermediate 18 had become available, saturation of the side chain and removal of the benzyl group were necessary as the next steps for access to 1c. Although debenzylation of (±)-23 had been accomplished by Nakaniishi’s group by hydrogenolysis over Pd–C in 2-propanol in the presence of acetic acid and hydrochloric acid with special care,\(^{11}\) we had smoothly removed the benzyl group from 1-benzyl-7-methylwyne (24a) by hydrogenolysis over Pd–C in methanol in the presence of aqueous perchloric acid.\(^{140}\) However, we previously experienced the formation of a by-product when 1-benzyl-7-(hydroxymethyl)wyne (24c) was subjected to hydrogenolysis under similar conditions, probably owing to acid-catalyzed generation of the stabilized carboxilation.\(^{140}\) To avoid the predictable formation of such a carboxilation we first saturated the side chain of 18 over 10% Pd–C in the absence of acid. The reduction was continued by addition of perchloric acid to afford 1c as the monohydrate \([\alpha]_D^{\text{25}} -45^\circ (\text{MeOH})\) in 75% yield. The UV, mass (MS), and \(^1H\)-NMR spectra of 1c thus obtained were identical with those of (±)-1c\(^{10,33}\) confirming the correctness of the structure in a two-dimensional sense. We have already assigned the 1,4-dihydro structure [N(1)-H tautomer] to 1a rather than the alternative 3,4-dihydro structure [N(3)-H tautomer] on the basis of UV spectral comparison with model compounds.\(^{34}\) This assignment has been supported by recent fluorescence studies on 1a and related compounds.\(^{35}\) The UV spectrum of 1c determined in 95% aqueous ethanol resembles that of (±)-1-benzylwybutine \([\alpha]_D^{\text{23}}\) rather than that of 3-β-D-ribofuranosylwybutine (25),\(^{36}\) suggesting that 1e also exists as the 1,4-dihydro structure [N(1)-H tautomer].

The identity of wybutine had been established by comparison of the MS\(^{70}\) and \(^1H\)-NMR spectra\(^{7,37}\) with those of (±)-1c.\(^{10}\) Further evidence in support of the identity was provided by direct comparison of wybutine\(^{38}\) with the present sample of synthetic 1c by means of high-performance liquid chromatography (HPLC). Although comparison of the CD spectrum of wybutine\(^{39}\) with that of synthetic 1c enabled us to assign an S configuration to wybutine, the intensity of the Cotton effect at 235 nm reported for wybutine was only a half of that of the present sample of 1c. Direct comparison of the CD spectra of natural\(^{38}\) and synthetic 1c revealed that they were superimposable except for the larger intensity (1.2 times) of the latter. The same intensity relationship was also recognized in the UV spectra of natural and synthetic 1c; the low potency of the natural sample was probably due to purification difficulties because of the minute amount available, as had already been noted.\(^{10}\) Although these results suggested that synthetic 1c was equivalent to the natural one in optical purity, both samples were ultimately demonstrated to be enantiomERICally pure by means of HPLC using a chiral column.

In conclusion, we have established a new method for the synthesis of optically active \(\beta\)-unsaturated \(\alpha\)-amino acid derivatives by use of the phosphonium chloride 6 as a synthetic equivalent for the nucleophilic alanine synthon.\(^{40}\) This method enabled us to perform the first chiral synthesis of 1c and to assign an S configuration to wybutine.

**Experimental**

**General Notes** All melting points were taken on a Yamato MP-1 capillary melting point apparatus and are corrected. Spectra reported herein were recorded on a Hitachi 320 UV spectrophotometer, a JASCO J-500C spectropolarimeter equipped with a JASCO DP-500N data processor, a Hitachi M-80 mass spectrometer, and a JEOL JNM-FX-100 NMR spectrometer at 25 °C with tetramethylsilane as an internal standard. Optical rotations were measured with a JASCO DIP-181 polarimeter using a 1-dm sample tube. The liquid chromatographic system was a Waters model 204 ALC which included a 6000A pump, a U6K injector, and a model 440 absorbance detector operating at 254 nm. Microanalyses were determined by Mr. Y. Itatani and his associates at Kanazawa University. Pre-coated silica gel plates (0.25 mm) with a fluorescent indicator (Merck) were used for analytical thin-layer chromatography (TLC). Flash chromatography was performed on silica gel according to the reported procedure.\(^{24}\) The following abbreviations are used: br = broad, d = doublet, dd = doublet-of-doublets, ddd = doublet-of-doublets-of-doublets, dt = doublet-of-triplets, m = multiplet, q = quartet, s = singlet, sh = shoulder, t = triplet.

(S)-\(N\)-(Methoxy carbonyl)serine Methyl Ester (3a) Methyl chlorofor- mate (11.3 g, 120 mmol) was added to a pre-cooled (10°C) solution of (S)-serine methyl ester hydrochloride (2a: X = –CH\(_2\)) (15.6 g, 100 mmol) in water (250 ml) in the presence of sodium bicarbonate (25 g) over a period of 5 min with vigorous stirring on a magnetic stirrer. The mixture was stirred at room temperature for a further 5 min, brought to pH 6 with 10% hydrochloric acid, and concentrated \(\textit{in vacuo}\) to ca. 70 ml. The resulting solution was extracted with dichloromethane (8 × 50 ml). The
combined organic layers were dried over magnesium sulfate and then concentrated in vacuo to leave a colorless heavy oil (16.7% 93%). [\{1\}]

(S)-N-(Methoxycarbonyl)benzyl ethyl ester p-toluenesulfonate (2b, X = TsO), which was prepared from (S)-serine (25.27 g, 240 mmol) according to the reported procedure, was dissolved in water (600 ml). Sodium bicarbonate (60.0 g, 714 mmol) and methyl chloride (22.3 ml, 256 mmol) were subsequently added to this solution under cooling in ice water over a period of 10 min, with vigorous stirring. Stirring was continued for a further 1.5 h at room temperature. The resulting oily precipitate crystallized on being kept in a refrigerator. The crystals were collected by filtration and dried to give a first crop of 3b (43.45 g), mp 39–44°C. A second crop (3.27 g, the total yield was 76% based on (S)-serine) was obtained from the mother liquor with dichloromethane (3 x 200 ml) followed successively by drying over magnesium sulfate and concentration in vacuo. Recrystallization from 50% (v/v) aqueous methanol followed by drying over phosphorous pentoxide at 2 mmHg and room temperature for 18 h gave an analytical sample as colorless, oil, mp 42–44°C (Found: C, 77.8; H, 7.5; N, 3.2; Anal. Calc. for C12H11NO3: C, 76.9; H, 7.2; N, 3.7). 1.80 mmol of (S)-N-(Methoxycarbonyl)benzyl ethyl ester p-toluenesulfonate (2b, X = TsO) was dissolved in dry pyridine (29 ml) at -10°C over a period of 20 min with stirring. The mixture was stirred for a further 3.5 h at -10°C then poured onto crushed ice (170 ml). The solid that separated was collected by filtration, washed with cold water (50 ml), and dried to give 8.84 g (85% yield), mp 83–85°C. Recrystallization from ethanol afforded an analytical sample of colorless prisms, mp 87–88°C. [\{2\}]

A solution of 3b (10.34 g, 58.5 mmol) in dry pyridine (29 ml) at -10°C over a period of 20 min with stirring. The mixture was stirred for a further 3.5 h at -10°C then poured onto crushed ice (170 ml). The solid that separated was collected by filtration, washed with cold water (50 ml), and dried to give a slightly brown solid (32.08 g, 81%), mp 75–83°C. Recrystallization from ethanol (30 ml) gave colorless needles (25.80 g, 65% yield, mp 89–90°C). Further recrystallization from ethanol gave an analytical sample of colorless prisms, mp 91°C. [\{3\}].

A solution of 3b (10.34 g, 58.5 mmol) in dry pyridine (29 ml) at -10°C over a period of 20 min with stirring. The mixture was stirred for a further 3.5 h at -10°C then poured onto crushed ice (170 ml). The solid that separated was collected by filtration, washed with cold water (50 ml), and dried to give a slightly brown solid (32.08 g, 81%), mp 75–83°C. Recrystallization from ethanol (30 ml) gave colorless needles (25.80 g, 65% yield, mp 89–90°C). Further recrystallization from ethanol gave an analytical sample of colorless prisms, mp 91°C. [\{3\}].

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2 mM Hg and 80 °C for 21 h to afford 6 (17.20 g, 98%) as a colorless glass, [x]D +57.1° (c = 0.50, CHCl3). [H-NMR (CDCl3) δ: 3.30 (3H, s, Me), 3.70–4.50 (3H br, CH2, CH), 7.50 (1H, br, NH), 7.60–8.00 (15H, m, PhH). This sample was of ca. 95% purity: the [H-NMR spectrum indicated that it was contaminated by a small amount of benzene and a trace of ethanol.

(S)-2-[[Homophenylalanine (10): Methyl chloroformate (0.04 mmol, 0.48 mmol)] was added to an ice-cooled mixture of (S)-2-aminophenylbutanoic acid (10) (54 mg, 0.30 mmol) and sodium carbonate (95 mg, 0.90 mmol) in water (6 mL). The solution was stirred at 0 °C for 10 min and then at room temperature for 2 h. The resulting mixture was brought to pH 7.0 and added to 10% H2O2 (0.2 mL) at room temperature. The resulting mixture was filtered and the filtrate was concentrated in vacuo to afford 12 (43.8 mg, 53.5%). The crude acid was then recrystallized from a mixture of ethyl acetate and hexane (3:1) and dried in a vacuum of 100 mm Hg at room temperature. This procedure was repeated three times, and the final filtrate and washings were concentrated in vacuo and the residue was dissolved in a mixture of methanol (0.1 mL) and benzene (5 mL).

Triethylmethylsilazidomethane (10% in hexane) (2 mL) was added to this solution and the mixture was concentrated in vacuo. The oily residue was purified by flash chromatography (hexane-ethyl acetate 2:1) to afford 14 (100 mg, 15%), [x]D +1.6° (c = 0.37, MeOH). This was identical (IR and H-NMR spectra) with 14 prepared by method (i).

[S-(E)-2-[1-Benzyl-4,9-dihydro-4,6-dimethyl-9-oxo-1H-imidazo[1,2-a]purin-7(6H)]-2-[methoxy carbonyl] amino]-3-butoenic Acid Methyl Ester (13) The phosphonium chloride (6.35 mmol) was gently stirred with methanol (40 mL) in a mixture of crystalline HMPA (3 mL) under argon at 30 °C for 23 h. The supernatant of the mixture (96 mL) was withdrawn with a syringe and transferred to a reaction vessel through a funnel with a fritted disk under argon. The solution was cooled to −78 °C and n-butyllithium (1.55 mol solution in hexane, 5.70 mL, 8.84 mmol) was added over a period of 1 h with stirring, and then 16 (60 mg, 0.44 mmol) was added over a period of 1 h and then allowed to warm to −18 °C over a period of 6 h. The resulting mixture was neutralized with 10% aqueous phosphoric acid and concentrated in vacuo to a small volume. After addition of water (40 mL), the mixture was brought to pH 3 by addition of 10% aqueous phosphoric acid and extracted with chloroform (4 x 30 mL). The organic layers were combined and concentrated under reduced pressure. The residue was suspended in water (20 mL) and then dissolved in a mixture of methanol (6 mL) and benzene (21 mL).

Triethylmethylsilazidomethane (1.5 mol solution in ether, 2.10 mL, 3.2 mmol) was added to the solution and then acetic acid (0.6 mL) was added after a delay of 1 min. The resulting mixture was concentrated in vacuo and purified by flash chromatography (column diameter, 30 mm; ethyl acetate-hexane 2:1) to afford 18 (221 mg, 16%) as a slightly yellow, waxy solid. Recrystallization from methanol slightly yellow needles (158 mg, 11%), mp 179–181 °C; [x]D +5.69° (c = 0.181, MeOH). Further recrystallization from methanol afforded a white analytical sample with unchanged melting point, [x]D +58.0° (c = 0.207, MeOH); UV (in the text). MS m/z: 464 (M+), 405 (M-162), 373 (M-180), 234 (M-222), 191 (M-255), 84 (M-308), 57 (M-380), 40 (M-450), 29 (M-560), 18 (M-670), 9 (M-780), 8 (M-890), 7 (M-990), 6 (M-1080), 5 (M-1190). The solution was filtered off and the filtrate was saturated with sodium carbonate. The mixture was concentrated in vacuo and the residue was treated with water (1 mL). The resulting precipitate was collected by filtration after storage of the mixture in a refrigerator overnight, washed with water (2 mL), and dried. It was dissolved in hot methanol (3 mL) and
insoluble material was removed by filtration while the solution was hot. The solution was concentrated in vacuo to give 1e (79 mg), mp 179–184°C (dec). The catalyst was extracted with methanol using a Soxhlet extractor. The methanolic solution was concentrated in vacuo and the residue was purified by thin-layer chromatography on silica gel (0.5 mm) [ethyl acetate–ethanol (10:1, v/v)] to afford a second crop of 1e (12 mg; the yield was 75% as the monohydrate). Recrystallization from methanol gave colorless needles, mp 208–211°C (dec). This sample was dried over diphosphorus pentoxide at 2 mm Hg and 80°C for 6 h and then exposed to air until a constant weight was reached to give an analytical sample as the monohydrate with unchanged ultraviolet (λmax = 332–450 nm) and infrared (3260 cm⁻¹) bands. Anal. Calcd for C₂₃H₂₇Cl₂N₄O₅S: C, 52.61; H, 5.23; N, 9.05%. Found: C, 52.49; H, 5.35; N, 8.99.}

References and Notes

9) References cited in ref. 14a.


37) The solvent, which is not specified in the literature, should be CDCl₃, according to a private communication from the senior author.

38) A very generous sample (colorless needles) was provided by Professor H. G. Zachau.
