Tetrahedron Vol. 46, No. 6, pp. 1975-1986, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

NITRILE OXIDES IN MEDICINAL CHEMISTRY--2‡. SYNTHESIS OF THE TWO ENANTIOMERS OF DIHYDROMUSCIMOL

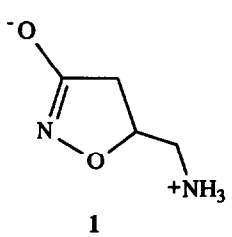
Marco De Amici, Carlo De Micheli* and Valeria Misani
Istituto Chimico-Farmaceutico - Viale Abruzzi,42 - 20131 Milano (Italy)

(*Received in UK* 21 *October* 1989)

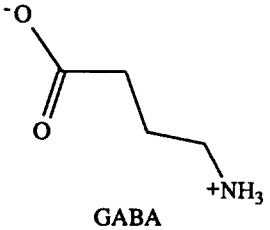
Abstract. *The cycloaddition of bromonitrile oxide to monosubstituted olefins has a high regioselectivity yielding 3-bromo-5-substituted isoxazolines contaminated by minor amounts (4-9%) of the 4-substituted isomer. The adducts of bromonitrile oxide to allyl a3lcohol and N-protected allylamine were employed as key intermediates in the preparation of racemic dihydromuscimol (DHM). The synthesis of (R)-(-)- and (S)-(+)-DHM was accomplished by using the two diastereomers obtained by the cycloaddition of bromonitrile oxide to (S)-(+)-isopropylidene-3-buten-1,2-diol. The enantiomeric excesses of (R)-(-)- and (S)-(+)-DHM, determined by capillary GLC on the appropriate precursors, were 98.8 and >99.0 %. A spectroscopic survey of the tautomerism of 3-hydroxyisoxazolines indicates the predominant or exclusive occurrence of the NH form.*

INTRODUCTION

5-aminomethylisoxazolin-3-ol **1** (dihydromuscimol - DHM) is a conformationally restricted analogue of the physiological neurotransmitter 4-aminobutyric acid (GABA).[1] Extensive pharmacological investigations of DHM have shown the existence of a powerful agonistic activity at the post-synaptic GABA receptor complex, associated with a significant interaction with the GABA-uptake system.[2] Recently, Krogsgaard-Larsen et al. have demonstrated that the inhibitory effects of DHM on the GABA-uptake system reside exclusively in its (R)-enantiomer, whereas the GABA-mimetic activity at the post-synaptic receptors is due to the (S)-enantiomer.[3]



1



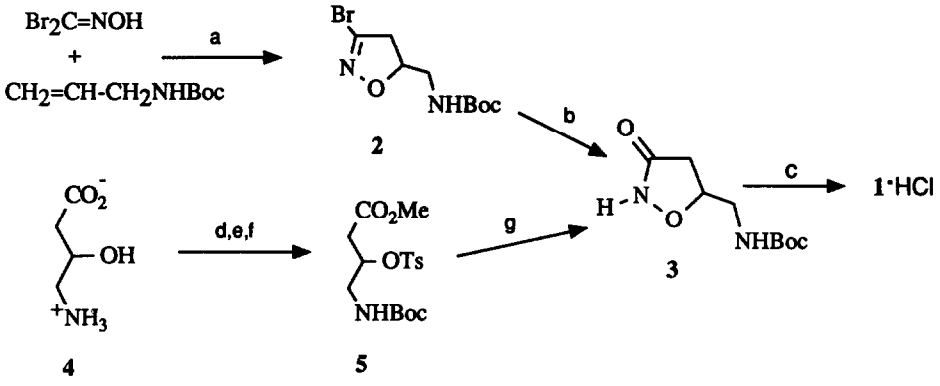
GABA

As part of our continuing study on the use of nitrile oxides in the synthesis of biologically active compounds,[4-7] we now report the synthesis of racemic DHM and an efficient preparation of the two enantiomers of DHM in high enantiomeric excess. Our strategy is based on the 1,3-dipolar cycloaddition of bromonitrile oxide to an appropriate dipolarophile.[8] As shown in Scheme 1, this methodology simplifies the previously reported route[9] to (±)-DHM, which used racemic aminoacid **4** as starting material. Furthermore, attempts to prepare the individual enantiomers of DHM by employing the corresponding chiral forms of **4**

‡Part 1: De Amici, M.; De Micheli, C.; Carrea, G.; Spezia, S. *J.Org.Chem.* **1989**, *54*, 2646.

were unsuccessful due to a complete racemization of intermediate **5** in the subsequent cyclization step.[10] (S)-(+)-, and (R)-(-)-DHM were then obtained via resolution of the diastereomeric salts of chinchonidine with the weakly acidic N-(Boc)DHM **3**.[3] Very recently an analogous result was also achieved by resolving racemic 3-chloro-5-aminomethyl-Δ^2-isoxazoline with (S)-(+)-mandelic acid.[11]

Scheme 1

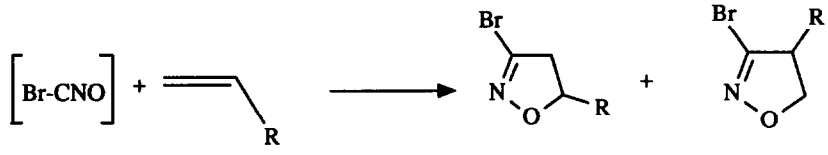


a: $NaHCO_3$, ethyl acetate; b: NaOH, THF-H_2O; c: HCl, ethyl acetate; d: MeOH, HCl; e: Boc_2O, NEt_3; f: TosCl, Py; g: MeONa, $NH_2CONHOH$.

RESULTS

As a preliminary study, we considered the regiochemistry of the cycloaddition of bromonitrile oxide to monosubstituted dipolarophiles, since Wade et al.[12] showed that the corresponding cycloadditions of chloronitrile oxide yielded 3-chloro-5-substituted isoxazolines contaminated by the 4-substituted regioisomers. We also investigated the tautomerism of 3-hydroxyisoxazolines, which can exist in the OH and/or in the NH form.

Scheme 2



	6	**7**
a: R = Ph	96	4
b: R = CH_2Cl	94	6
c: R = C_4H_9	93	7
d: R = CH_2OH	91	9

The data collected in Scheme 2 evidence that bromonitrile oxide behaves like chloronitrile oxide, producing a mixture of the two regioisomers, in all the examined cases. The presence of both the regioisomers

in all the cycloadditions of cyanogen halogenide N-oxides to monosubstituted olefins and acetylenes[13,14] will be studied by means of semiempirical calculations and will be reported in due course.

The reported ratios were evaluated by 200 MHz ^{1}H NMR analyses and for styrene was confirmed by a capillary GLC determination. Table I reports the relevant ^{1}H NMR data for isoxazolines **6a-d**, and **7a-d**. In both the regioisomers, protons attached to the 5-position of the heterocyclic ring resonate at a lower field than those linked to the 4-position; as expected the difference in chemical shift is smaller for the 4-substituted regioisomers **7a-d**. The sole adduct **7a**, due to the deshielding effect of the phenyl ring, shows H-4 and H-5'at the same chemical shift. It is worth pointing out the different values of the geminal coupling constant for the two regioisomers. Due to the influence of the heterocyclic oxygen, $J_{4,4'}$ of **6a-d** is substantially larger than $J_{5,5'}$ of **7a-d**.

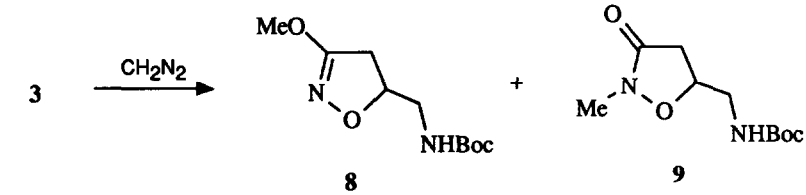
Table I: ^{1}H NMR data of Isoxazolines **6** and **7**. Chemical shifts in δ; coupling constants in Hz.

compd.	H-5	H-4	H-4'	$J_{4,5}$	$J_{4',5}$	$J_{4,4'}$
6a	5.67	3.61	3.22	10.7	9.0	17.3
6b	4.92	3.38	3.20	10.5	7.0	17.5
6c	4.66	3.24	2.84	10.6	8.7	17.1
6d	4.80	3.27	3.18	10.0	9.0	17.5
	H-4	H-5	H-5'	$J_{4,5}$	$J_{4,5'}$	$J_{5,5'}$
7a[a]	4.43	4.82	4.42	11.3	7.2	8.7
7b	[b]	4.60	4.46	10.2	7.4	8.5
7c	3.42	4.50	4.12	10.5	8.2	8.2
7d	3.50	4.57	4.45	10.7	8.2	8.2

[a]The spectrum was simulated with the LAOCN3 program as an ABC system; [b]Buried under other signals.

The tautomerism of 3-hydroxyisoxazolines was studied on N(Boc)-DHM **3**, by comparing its UV and IR spectra with those of 3-methoxy derivative **8** and N-methyl derivative **9** (Scheme 3).

Scheme 3



λ^a_{max}	ε	λ^a_{max}	ε	λ^a_{max}	ε
219	3680	215.5	2895	219	3700

[a] Value in nm, determined in abs. ethanol.

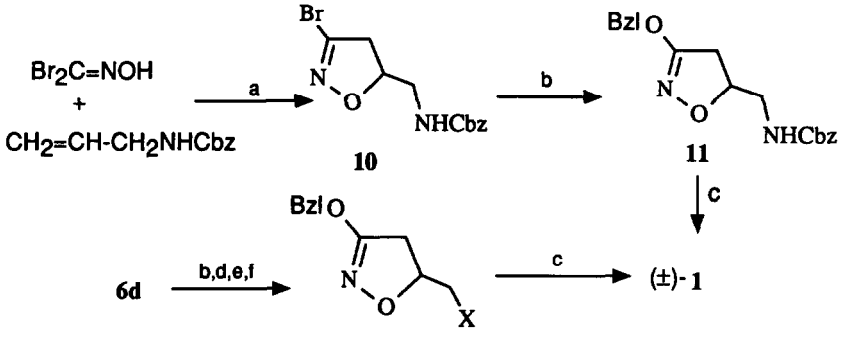
Compounds **8** and **9**, prepared in comparable amounts by reacting **3** with an ethereal solution of diazomethane, were separated by column chromatography and characterized by ^{1}H NMR. Adduct **8** was also

prepared (92% yield) by exchanging a methoxy group for the 3-bromo substituent of **2** under basic conditions (barium hydroxide) in a 5:1 MeOH-water mixture.

The similarity of the UV spectra of **3** and **9** (Scheme 3), in comparison with the slight difference from that of **8**, seems to indicate the predominant or exclusive occurrence of the NH form, for derivative **3**, at least in organic non-polar solvents. This conclusion is confirmed by the presence in its IR spectrum of an NH absorption at 3413 cm^{-1} and by the concomitant absence of the C=N stretching band (1626 cm^{-1} for **8**). We could not utilize the C=O absorption due to the presence of the NHBoc group. For this reason throughout this paper the structures of 3-hydroxyisoxazolines will be reported in the NH form. We are not aware of the existence of any previous studies on the tautomerism of 3-hydoxyisoxazolines,[15] but this isomerism has been considered for 3-aminoisoxazolines.[16] Physicochemical investigations (NMR, UV and IR) in both solution and the solid state allowed the detection of the sole 3-amino tautomer.[16]

The cycloaddition of bromonitrile oxide to N(Boc)allylamine was carried out under heterogeneous conditions and yielded 3-bromoisoxazoline **2**, in almost quantitative yield, which was purified from the 4-substituted regioisomer (7.5%) by crystallization. Synthesis of the key intermediate **3** was achieved in 68% yield by heating a sodium hydroxide solution of **2** under biphasic conditions (THF-water) in the presence of a phase-transfer catalyst (tetrabutylammonium hydrogensulfate) at 60°C. In order to find milder conditions and also to introduce the hydroxy group in a protected form, we considered the two reaction sequences reported in Scheme 4. Both these strategies proved to be successful and produced (±)-DHM in satisfactory overall yields

Scheme 4



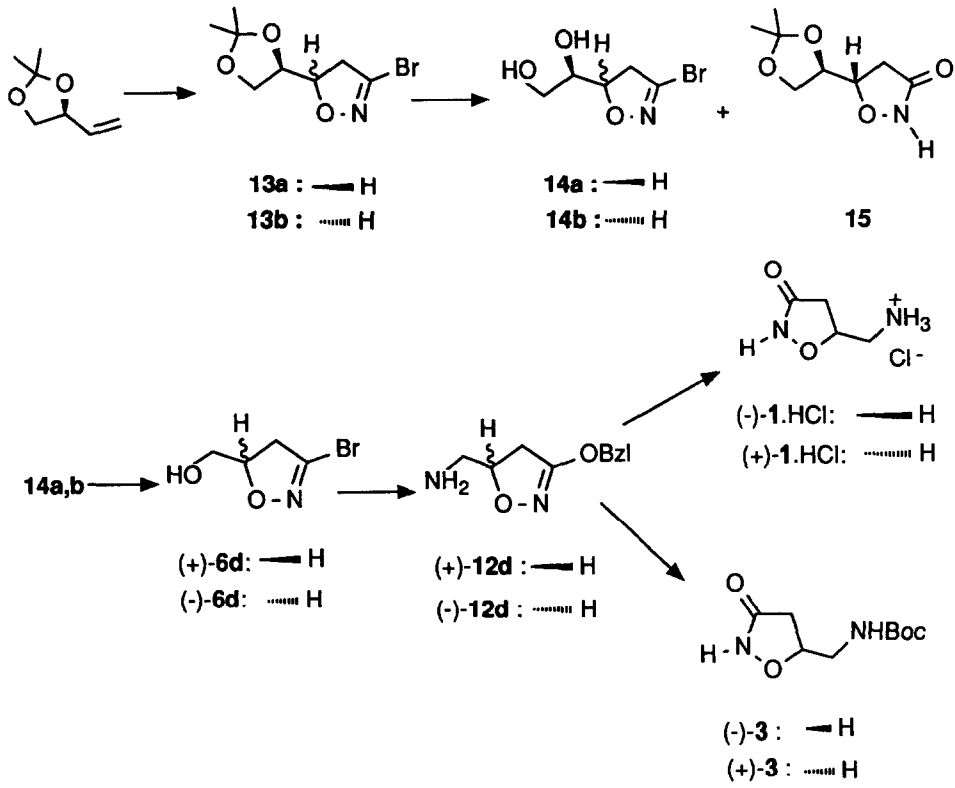
12a: X = OH
12b: X = OMs
12c: X = N_3
12d: X = NH_2

a: $NaHCO_3$, ethyl acetate; b: BzOLi-DMSO; c: HCl, ethyl acetate; d: $MeSO_2Cl$-NEt_3; e: NaN_3-DMSO; f: Ph_3P,THF-H_2O.

(48 and 31%). The presence of small amounts of the 4-substituted regioisomer does not hinder the synthesis of (±)-DHM, since some of the intermediates can be crystallized. The displacement of the 3-bromo substituent of **10** and **6d** by the benzyloxy anion was efficiently accomplished at room temperature in a DMSO solution.[17] We considered the sequence which uses 3-bromo-5-hydroxymethylisoxazoline **6d** to be a suitable model to extend to the preparation of the two enantiomers of DHM. For such a purpose we cycloadded bromonitrile oxide to (S)-(+)-isopropylidene-3-buten-1,2- diol[18] to yield the 5-substituted

diastereomers *anti* **13a** and *syn* **13b** in a 76:24 ratio[19] as the sole detectable regioisomer, and in almost quantitative yield (Scheme 5). As expected on the basis of experimental and theoretical results for related models,[20] *anti* isomer (i.e. **13a**) is always predominant. The structural assignments are based on ^{1}H NMR analyses; as previously reported for related systems,[21] the $J_{5,\alpha}$ coupling constant is always larger for the *anti* isomers (i.e. **13a**-**13b** 5.1 vs. 4.1 Hz). Compounds **13a** and **13b** were separated by column chromatography and then converted into enantiomers (R)-(-)-**6d** and (S)-(+)-**6d** via a sequence of steps (30-35% overall yield) involving IRA-120 promoted acetonide cleavage, sodium periodate oxidative cleavage of the intermediate diols **14a,b**, and sodium borohydride reduction.

Scheme 5



It is worth pointing out that transketalization of **13a** produced, besides the expected diol **14a**, a minor amount (8.5%) of derivative **15** which, reasonably, derives from the reaction of the substrate with the moisture present in the polymeric catalyst. This result is surprising when we consider that 3-bromoisoxazolines (i.e. **6d**) failed to react under identical conditions (IRA-120 and methanol). Derivative **15** was characterized by ^{1}H NMR and mass spectra. The same reaction carried out on **13b** did not produce an appreciable amount of the corresponding diastereomer of **15**. The enantiomeric excess of (R)-(-)-**6d** and (S)-(+)-**6d** was carefully determined by capillary gas chromatographic analyses of the corresponding Mosher's esters (98.8 and >99.0%). Enantiomers (R)-(-)-**6d** and (S)-(+)-**6d** were then transformed into the

related 3-benzyloxy derivatives (+)-**12d** and (-)-**12d** with the sequence of steps previously described for the racemic form. The synthesis of (+)-**1**.HCl and (-)-**1**.HCl was finally achieved through a catalytic hydrogenation followed by treatment with HCl in ethyl acetate. Specific rotations of the final derivatives (+)-**1**.HCl and (-)-**1**.HCl are in perfect agreement with the data previously published for the same compounds.[3] Further evidence for the optical purity of the compounds was obtained by matching the optical rotations of (+)-**3** and (-)-**3** with the data reported in the literature.[3] These derivatives were obtained by reacting (+)-**12d** and (-)-**12d** with Boc_2O followed by a catalytic hydrogenation over 5% Pd/C.

To conclude, a suitable preparation of both the enantiomers of DHM, via the 1,3-dipolar cycloaddition of bromonitrile oxide to a chiral dipolarophile, was accomplished in a remarkable enantiomeric excess and reasonable chemical yield .

Experimental Section

Material and Methods - Styrene, allyl chloride, 1-hexene, and allyl alcohol were purchased from Fluka and used as such. (S)-(+)-isopropylidene-3-buten-1,2-diol[18] and dibromoformaldoxime[22] were prepared according to the referenced literature methods. ^1H NMR spectra were recorded in $CDCl_3$ solution at 80 or 200 MHz. GLC analyses were carried out on a Carlo Erba HRGC 5100 Mega Series gas chromatograph equipped with a Supelco SP-2340 capillary silica gel column (30 m, 0.2 μm); H_2 was used as the carrier gas at 0.5 atm. Rotatory power determinations were carried out with a Perkin-Elmer 241 polarimeter, coupled with a Haake N3-B thermostat. UV spectra were recorded on a Beckman model 24 spectrophotometer in abs. ethanol. IR spectra were performed on a Perkin Elmer 983 spectrometer coupled with a Perkin Elmer 3600 Data Station in carbon tetrachloride at a concentration of 1.45×10^{-2}M. Mass spectra were measured on a Finnigan MATT 8222 using the chemical ionization mode (isobutane). Merck silica gel 60 F_{254} analytical thin-layer chromatography plates were used throughout this work. Microanalyses (C, H, and N) of new products agreed with theoretical value ±0.3%. (R)-(+)-MTPA esters were prepared according to the procedure previously described[20]. Liquids were characterized by the oven temperature for Kugelrohr distillations.

N(*tert.*Butoxycarbonyl)allylamine. To a magnetically stirred and ice-cooled solution of di-*tert.*butyldicarbonate (6.36 g, 29.2 mmol) in dichloromethane (30 mL), a CH_2Cl_2 solution (20 mL) of allylamine (2 g, 35 mmol) was added dropwise. The mixture was stirred overnight at room temperature then the solvent was removed under vacuum. The residue (3.94 g, 86% yield) crystallizes from ligroin as colorless prisms: mp 35-36°C; ^1H NMR δ 1.40(s, 9H, 3Me), 3.70(m, 2H, CH_2N), 4.80-4.20(bs, 1H, NH), 5.18(m, 2H, CH_2=), 5.85(m, 1H, CH=).

N(Benzyloxycarbonyl)allylamine. Benzyl chloroformate (6.82 g, 40 mmol) was added to a magnetically stirred and ice-cooled suspension of allylamine (3.0 g, 52.5 mmol) and sodium carbonate (10.6 g, 0.1 mol) in dry acetone (20 mL). The mixture was refluxed until carbon dioxide evolution ceased (6 h). The solvent and excess allylamine were removed at reduced pressure; the residue was poured into water and extracted with dichloromethane. The organic extracts were dried over anhydrous sodium sulfate, the solvent

was removed at reduced pressure, and the residue (5.65 g, 74% yield) Kugelrohr distilled at 120-125°C/1 mmHg; ^{1}H NMR δ 3.80(m, 2H, CH_2N), 5.12(s, 2H, OCH_2), 4.90-5.40(m, 3H, CH_2=, and NH), 5.78(m, 1H, CH=), 7.35(s, 5H, arom.).

Standard Procedure for the Preparation of 3-Bromo-Δ^2-isoxazolines 2, 6a-d and 7a-d, 10. The following procedure is representative. A 100 mL Erlenmeyer flask was loaded with the followings: ethyl acetate (50 mL), dibromoformaldoxime[22] (2.7 g, 13.0 mmol), solid sodium bicarbonate (4.2 g, 50.0 mmol), N(Boc)allylamine (1.8 g, 11.5 mmol). The reaction mixture was magnetically stirred at room temperature until evolution of gas ceased then poured into water and extracted with ethyl acetate (3x15 mL). The organic extracts were dried over anhydrous sodium sulfate and the solvent was removed under vacuum. The crude adduct (2.92 g, 91% yield) crystallizes from ligroin/ethyl acetate as colorless prisms: mp 69-70°C; Found: C% 38.52; H% 5.64; N% 10.05. $C_9H_{15}BrN_2O_3$ requires: C% 38.72; H% 5.42; N% 10.04; ^{1}H NMR(DMSO) δ 1.45(s, 9H), 3.01(dd, 1H, J= 7.8, 17.6 Hz), 3.23(dd, 1H, J= 10.7, 17.6 Hz), 3.37(m, 2H, CH_2N), 4.75(m, 1H), 4.86(bs, 1H).

A ^{1}H NMR of the crude reaction mixture showed the presence of 7.5% of the 4-substituted regioisomer. ^{1}HNMR(DMSO) δ 3.48(m, 1H), 4.30(dd, 1H, J= 7.8, 9.0 Hz), 4.51(dd, 1H, J= 9.0, 10.5 Hz).

With the same procedure, N(benzyloxycarbonyl)allylamine (1.43 g, 7.5 mmol) was reacted with dibromoformaldoxime (1.38 g, 6.8 mmol) to give crude adduct **10** (1.83 g, 86% yield) which crystallizes from ligroin/ethyl acetate as colorless prisms: mp 72.5-73°C; Found: C% 46.25; H% 4.07; N% 8.91. $C_{12}H_{13}BrN_2O_3$ requires: C% 46.02; H% 4.18; N% 8.95; ^{1}H NMR δ 3.15(m, 2H, H-4), 3.50(m, 2H, CH_2N), 4.82(m, 1H, H-5), 5.17(s, 2H, CH_2O), 7.40(s, 5H, arom.).

The cycloadditions of bromonitrile oxide to styrene, allyl chloride, 1-hexene, and allyl alcohol were carried out under identical conditions with a 1,3-dipole/dipolarophile ratio of 1:5. The yields are higher than 80% and the ratios **6a-d/7a-d** reported in Scheme 2 were obtained by ^{1}H NMR analysis of the crude reaction mixtures; in the case of **6a/7a** the ratio was confirmed by capillary gas chromatography under the following conditions: carrier H_2, 1.2 mL/min; 40°C (1 min) to 230°C (25 min), heating rate 10°/min. **6a**: 51.6 min; **7a**: 48.9 min. **6a**: bp 100°C/0.1 mmHg; **6b**: bp 105°C/0.3 mmHg; **6c**: bp 90°C/0.3 mmHg; **6d**: bp 160°C/1 mmHg.

(R,S)-5-N(*tert*.Butyloxycarbonyl)aminomethyl-3-oxo-isoxazolidine 3. To a solution of **2** (0.6 g, 2.15 mmol) in THF (15 mL) were added an aqueous solution of sodium hydroxide (60 mL, 1N) and 60 mg of tetrabutylammonium hydrogensulfate. The resulting mixture was magnetically stirred and heated at 60°C until TLC (eluent: ethyl acetate) showed disappearance of the starting material (5 h). The reaction mixture was extracted with ether and the aqueous phase made acid with dil. HCl. After extraction (4x20 mL CH_2Cl_2) and evaporation of the solvent, the residue (0.315 g, 68% yield) was crystallized from n.hexane/ethyl acetate 4:1 to give **3** as colorless prisms. mp 103-108°C (lit.[3] 89-90°C); Found: C% 50.00; H% 7.47; N% 12.90. $C_9H_{16}N_2O_4$ requires: C% 49.99; H% 7.46; N% 12.95; ^{1}H NMR δ 1.47(s, 9H, CMe_3), 2.70(m, 2H, H-4), 3.44(m, 2H, CH_2N), 4.68(m, 1H, H-5), 5.10(bs, 1H, CH_2NH), 8.30(bs, 1H, NH); UV(abs EtOH) λ_{max} 219, ε= 3682; IR(CCl_4) ν(cm^{-1}) 3460, 3415, 1715.

(R,S)-5-Aminomethyl-3-oxo-isoxazolidine hydrochloride 1.HCl. The title compound was prepared according to the protocol previously reported[9].

Reaction of 3 with diazomethane. A solution of **3** (0.108 g, 0.5 mmol) in dichloromethane (5 mL) was treated portionwise with a solution of diazomethane in ether. Excess diazomethane was destroyed with dil. HCl, the organic layer was washed with a saturated $NaHCO_3$ solution, then dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was column chromatographed (eluent: cyclohexane/ethyl acetate 1:4) to yield 57 mg of(±)-**8** (R_F= 0.76) and 48 mg of (±)-**9** (R_F= 0.42). Overall yield: 91.3%.

8 distilled at 140-145°C/0.8 mmHg; Found: C% 51.89; H% 7.69; N% 12.25. $C_{10}H_{18}N_2O_4$ requires: C% 52.16; H% 7.88; N% 12.17; ^{1}H NMR δ 1.47(s, 9H, CMe_3), 2.90(m, 2H, H-4), 3.38(m, 2H, CH_2N), 3.87(s, 3H, OMe), 4.73(m, 1H, H-5), 5.10(bs, 1H, NH); UV(abs EtOH) λ_{max} 215.5 ϵ= 2893.5; IR(CCl_4) ν(cm^{-1}) 3453, 1715, 1626.

9 distilled at 170-175°C/0.8 mmHg; Found: C% 51.93; H% 7.64; N% 12.35; ^{1}H NMR δ 1.47(s, 9H, CMe_3), 2.72(m, 2H, H-4), 3.15(s, 3H, OMe), 3.44(m, 2H, CH_2N), 4.62(m, 1H, H-5), 5.00(bs, 1H, NH); UV(abs EtOH) λ_{max} 219, ϵ= 3700; IR(CCl_4) ν(cm^{-1}) 3459, 1715.

(R,S)-5-N(*tert*.Butyloxycarbonyl)aminomethyl-3-methoxy-Δ^2-isoxazoline 8. A slurry of **2** (0.2 g, 1.0 mmol) and $Ba(OH)_2.8H_2O$ (3.0 g, 10 mmol) in MeOH-H_2O (15 mL, 5:1) was stirred at room temperature until disappearance of the starting material (2 h). After the usual work-up, **8** was recovered in 92% yield.

(R,S)-3-Benzyloxy-5-N(benzyloxycarbonyl)aminomethyl-Δ^2-isoxazoline 11. To a solution of benzyl alcohol (3.9 mL) in anhydrous DMSO (25 mL) was added butyl lithium (3.8 mL, 1.6 M in hexane). The suspension was stirred at room temperature for 15 min then poured into a solution of **10** (0.518 g, 1.65 mmol) in DMSO (5 mL). The mixture was stirred for 1 h, then poured onto ice and extracted with diethyl ether (3x15 mL). The organic extracts were washed with water (4x20 mL) then dried over anhydrous sodium sulfate. The solvent and excess benzyl alcohol (90°C/0.5 mmHg) were removed under vacuum; the residue (0.36 g, 65% yield) was crystallized from ligroin-ethyl acetate as colorless prisms, mp 85-86°C; Found: C% 67.21; H% 5.87; N% 8.20. $C_{19}H_{20}N_2O_4$ requires: C% 67.04; H% 5.92; N% 8.23; ^{1}H NMR δ 3.05(m, 2H, H-4), 3.61(m, 2H, CH_2N), 4.92(m, 1H, H-5), 5.27(s, 5H, $2CH_2O$ and NH), 7.50(s, 10H, arom.).

(R,S)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline (±)-**12a**. The above reported procedure was applied to (±)-**6d** (1.25 g, 6.95 mmol) to yield (±)-**12a** (0.75 g, 52% yield) as a colorless oil which was Kugelrohr distilled at 210-215°C/0.5 mmHg; ^{1}H NMR δ 2.40(bs, 1H, OH), 3.08(m, 2H, H-4), 3.78(bs, 2H, CH_2OH), 4.81(m, 1H, H-5), 5.20(s, 2H, CH_2O), 7.43(s, 5H, arom.).

(R,S)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline methanesulfonate. (±)-**12b**. A solution of (±)-**12a** (0.741 g, 3.58 mmol) and triethylamine (1.25 mL) in dichloromethane (15 mL) was stirred at -15°C under N_2 during the addition of methanesulfonyl chloride (400 μL). After the addition was completed, the solution was warmed and stirred at room temperature overnight. The solution was diluted with Et_2O, washed twice with dil. HCl, with brine then dried over Na_2SO_4. Removal of the solvent yielded a residue which was

purified by column chromatography on silica gel using 7:3 cyclohexane-ethyl acetate as eluent. Derivative (±)-**12b** (0.933 g, 91%) was crystallized from n.hexane-ethyl acetate as colorless needles; mp 91.5-93°C; Found: C% 50.35; H% 5.23; N% 4.99. $C_{12}H_{15}NO_5S$ requires: C% 50.51; H% 5.30; N% 4.91; ^{1}H NMR δ 2.95-3.20(m, 5H, CH_3 and H-4), 4.35(d, 2H, CH_2OSO_2, $J_{AX}+J_{BX}$=10.6 Hz), 4.92(m, 1H, H-5), 5.19(s, 2H, $ArCH_2O$), 7.45(s, 5H, arom.).

(R,S)-3-Benzyloxy-5-azidomethyl-Δ^2-isoxazoline (±)-**12c**. A solution of (±)-**12b** (0.35 g, 1.23 mmol) and sodium azide (0.480 g, 7.37 mmol) in DMSO (15 mL) was heated at 50°C for 1 h with stirring. The solution was poured into water and extracted with Et_2O (3x15 mL). The extracts were washed three times with water, dried over anhydrous Na_2SO_4, concentrated and column chromatographed (eluent: 20% ethyl acetate-cyclohexane) yielding 269 mg (94%) of (±)-**12c** as a colorless thick oil; IR ν(cm^{-1}) 2150; ^{1}H NMR δ 3.04(m, 2H, H-4), 3.47(d, 2H, CH_2N; $J_{AX}+J_{BX}$=12.0 Hz), 4.80(m, 1H, H-5), 5.19(s, 2H, CH_2O), 7.46(s, 5H, arom.).

(R,S)-3-Benzyloxy-5-aminomethyl-Δ^2-isoxazoline (±)-**12d**. A solution of (±)-**12c** (1.16 g, 5 mmol) and triphenylphosphine (1.5 g, 5.7 mmol) in THF(25 mL) and water (200 μL) was stirred at room temperature for 24 h. The solvent was removed at reduced pressure and the residue, taken up with dil. HCl, was extracted with CH_2Cl_2 (2x100 mL). The aqueous phase was made alkaline with K_2CO_3 then extracted with CH_2Cl_2(3x100 mL). The extracts were dried (Na_2SO_4) and concentrated yielding 0.81 g (78%) of (±)-**12d** which crystallized from ligroin as colorless prisms; mp 50-52°C; Found: C% 64.02; H% 6.68; N% 13.75. $C_{11}H_{14}N_2O_2$ requires: C% 64.06; H% 6.84; N% 13.58; ^{1}H NMR δ 1.45(bs, 2H, NH_2), 2.60-3.40(m, 4H, CH_2N and H-4), 4.68(m, 1H, H-5), 5.17(s, 2H, CH_2O), 7.44(s, 5H, arom.).

(R,S)-5-Aminomethyl-3-oxo-isoxazolidine (±)-**1**. A solution of (±)-**12d** (1.0 g, 4.8 mmol) in EtOH was hydrogenated over 5% Pd/C until absorption of one equivalent of hydrogen. Removal of the catalyst by filtration and evaporation of the solvent under vacuum gave a residue of (±)-**1** (0.523 g, 93%) as a thick yellowish oil; Found: C% 41.09; H% 7.21; N% 23.97. $C_4H_8N_2O_2$ requires: C% 41.37; H% 6.94; N% 24.13; ^{1}H NMR(D_2O) δ 3.11(m, 2H, H-4), 4.72(m, 1H, H-5), 5.30(bs, 3H, NH_3^+).

(±)-**1** was quantitatively transformed into the corresponding hydrochloride by treatment with HCl in ethyl acetate.

Hydrogenation of **11**, under identical conditions, yielded (±)-**1** in 85% yield which was quantitatively transformed into (±)-**1**.HCl by treatment with HCl in ethyl acetate.

Cycloaddition of bromonitrile oxide to (S)-(+)-isopropylidene-3-buten-1,2-diol. A 100 mL Erlenmeyer flask fitted with a magnetic stirring bar and a silicone-oil bubbler was charged with the followings: dibromoformaldoxime (5.5 g, 27 mmol), (S)-(+)-isopropylidene-3-buten-1,2-diol (3.2 g, 25 mmol), $NaHCO_3$ (10.5 g, 125 mmol), and ethyl acetate (40 mL). The mixture was stirred at room temperature for 24 h then filtered and the solids washed carefully with ethyl acetate. The combined organic fractions were concentrated at reduced pressure and submitted to column chromatography on silica gel (eluent: 15% ethyl acetate-cyclohexane). The adducts were recovered in the following order of elution: *anti* **13a** (4.51 g), *syn*

13b (1.50 g). A capillary GLC [carrier H_2 1.2 mL/min; 40°C (1 min) to 230°C (25 min), heating rate 10°C/min] of the reaction mixture gave a ratio **13a/13b** of 76:24[19]; **13a**: 19.4 min; **13b**: 20.5 min.

13a: R_F 0.51(silica gel, 25% ethyl acetate-cyclohexane) crystallized from ligroin as colorless needles; mp 71-72°C; Found: C% 38.41; H% 4.75; N% 5.47. $C_8H_{12}BrNO_3$ requires: C% 38.42; H% 4.84; N% 5.60; $[\alpha]^{20}_D$ +75.52°(c 1.35, $CHCl_3$); ^1H NMR($CDCl_3$) δ 1.33(s, 3H), 1.41(s, 3H), 3.20(dd, 1H, J=17.4, 10.5 Hz), 3.36(dd, 1H, J=17.4, 8.3), 3.64(dd, 1H, J= 11.3, 5.7 Hz), 3.76(dd, 1H, J= 3.7, 11.3 Hz), 3.89(m, 1H), 4.68(ddd, 1H, J= 5.1, 10.5, 8.3).

13b: R_F 0.40 colorless thick oil which solidifies at -20°C; Found: C% 38.29; H% 4.98; N% 5.69; $[\alpha]^{20}_D$ -114.97°(c 1.42, $CHCl_3$); ^1H NMR($CDCl_3$) δ 1.34(s, 3H), 1.41(s, 3H), 3.13(dd, 1H, J= 17.3, 8.5), 3.31(dd, 1H, J= 17.3, 10.0 Hz), 3.86(dd, 1H, J= 8.7, 6.0 Hz), 4.05(dd, 1H, J= 8.7, 8.7 Hz), 4.25(ddd, 1H, J= 4.1, 6.0, 8.7 Hz), 4.73(ddd, 1H, J= 8.5, 10.0, 4.1 Hz).

(5S)-5-[(1R)-1,2-Dihydroxyethyl]-3-bromo-Δ^2-isoxazoline 14a and (5S)-5-[(4R)-(2,2-Dimethyl--1,3-dioxolan-4-yl)]-3-oxo-isoxazolidine 15. A mixture of 3.68 g of **13a** and 0.30 g of IRA-120 resin in 70 mL methanol was stirred and heated at reflux for 1 h. The reaction mixture was filtered and concentrated at reduced pressure. Column chromatography of the residue on silica gel with 1:1 cyclohexane-ethyl acetate afforded 2.29 g (74%) of **14a** and 0.21 g (8.5%) of **15**. **14a** R_F 0.24 (silica gel 3:2 ethyl acetate-cyclohexane crystallized from diisopropyl ether-ethyl acetate as colorless prisms mp 58.5-60°C; Found: C% 28.81; H% 3.98; N% 6.54. $C_5H_8BrNO_3$ requires: C% 28.59; H% 3.84; N% 6.67; $[\alpha]^{20}_D$ +94.43°(c 1.01, $CHCl_3$); ^1H NMR($CDCl_3$) δ 2.70(bs, 2H), 3.12(dd, 1H, J= 18.1, 7.8 Hz), 3.42(dd, 1H, J=18.1, 11.1 Hz), 3.70-4.10(m, 3H), 4.67(ddd, 1H, J= 11.1, 7.8, 4.7 Hz).

15 distilled as colorless oil at 190-195°C/0.5 mmHg; $[\alpha]^{20}_D$ -12.84° (c 1.03 $CHCl_3$); ^1H NMR($CDCl_3$) δ 1.75(s, 3H), 1.77(s, 3H), 2.54(dd, 1H, J= 18.6, 2.0 Hz), 2.88(dd, 1H, J= 18.6, 7.2), 3.70(dd, 1H, J= 13.1, 3.0 Hz), 3.82(dd, 1H, J= 13.1, 2.9 Hz); 4.54(m, 1H), 4.74(ddd, 1H, J= 7.5, 2.0, 2.0 Hz); mass spectrum (CI isobutane), m/z 188(M^++1).

(5R)-5-[(1R)-1,2-Dihydroxyethyl]-3-bromo-Δ^2-isoxazoline 14b. The treatment of **13b** according to the above reported procedure gave **14b** in 77% yield. Derivative **14b**, R_F 0.17 (silica gel 3:2 ethyl acetate-cyclohexane), crystallized from diisopropyl ether/ethyl acetate as colorless prisms, mp 65.5-67°C; Found: C% 28.77; H% 4.06; N% 6.87; $[\alpha]^{20}_D$ -150.31(c 0.97, $CHCl_3$); ^1H NMR($CDCl_3$) δ 2.30(bs, 2H), 3.23(dd, 1H, J= 17.9, 8.8 Hz), 3.29(dd, 1H, J= 17.9, 10.4 Hz), 3.71(bs, 3H), 4.75(ddd, 1H, J= 10.4, 8.8, 3.3 Hz).

(5S)-3-Bromo-5-hydroxymethyl-Δ^2-isoxazoline (+)-6d. To a solution of 2.19 g (10.38 mmol) of diol **14a** in 250 mL of MeOH-H_2O (3:1) was added sodium periodate in water (52 mL - 0.23 M). After being stirred at room temperature for 25 min, the mixture was filtered through Celite. To the filtrate was added, portionwise, $NaBH_4$ (3.78 g) at ice-bath temperature. The mixture was stirred at that temperature for 30 min, and then 26 mL dil. HCl was added dropwise to destroy the excess reagent. The methanol was removed under vacuo, and the residue was extracted "in continuum" with dichloromethane. The extract was dried (Na_2SO_4) and concentrated to give an oily residue which was column chromatographed on silica gel with 7:3

cyclohexane-ethyl acetate; (+)-**6d** (1.23 g, 66%) was Kugelrohr distilled at 158-163°C/1 mmHg; this product, on standing at -25°C, turns crystalline: mp 36.5-38°C; Found: C% 26.44; H% 3.21; N% 7.81. $C_4H_6BrNO_2$ requires: C% 26.69; N% 3.36; H% 7.78; $[\alpha]^{20}_D$ +141.44°(c 0.934, $CHCl_3$).

(5R)-3-Bromo-5-hydroxymethyl-Δ^2-isoxazoline (-)-6d. This enantiomer was prepared, in 64% yield, from **14b** following the above reported procedure; Found: C% 26.52; H% 3.12; N% 7.94; $[\alpha]^{20}_D$ -138.81°(c 1.054, $CHCl_3$).

The spectral data of (+)-**6d** and (-)-**6d** are the same as reported for their racemic form.

Determination of enantiomeric excess of (+)-6d and (-)-6d. The two enantiomers of **6d** and the corresponding racemic form were converted into the (R)-(+)-MTPA esters[23] and submitted to GLC with a commercially available capillary column (SP 2340, 30 m, 0,2 μm) under the following conditions: 40°C(3 min) to 225°C(30 min), 5°C/min heating rate. Retention times (min): 55.6(S) and 57.3(R).

The transformation of (+)-**6d** and (-)-**6d** into (+)-**12d** and (-)-**12d** respectively was achieved with the sequence of steps previously described for the racemic form [(±)-**6d** to (±)-**12d**]..

(5S)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline (+)-12a: bp 210-215°C/0.5 mmHg; $[\alpha]^{20}_D$ +74.49°(c 0.592, $CHCl_3$).

(5R)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline (-)-12a: $[\alpha]^{20}_D$ -74.81°(c 0.798, $CHCl_3$).

(5S)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline methanesulfonate (+)-12b: colorless prisms from n.hexane-ethyl acetate; mp 75-75.5°C; Found: C% 50.44; H% 5.18; N% 4.81; $[\alpha]^{20}_D$ +84.55°(c 0.576, $CHCl_3$).

(5R)-3-Benzyloxy-5-hydroxymethyl-Δ^2-isoxazoline methanesulfonate (-)-12b: Found: C% 50.39; H% 5.37; N% 4.93; $[\alpha]^{20}_D$ -84.89°(c 0.622, $CHCl_3$).

(5S)-3-Benzyloxy-5-azidomethyl-Δ^2-isoxazoline (+)-12c: colorless thick oil; $[\alpha]^{20}_D$ +166.93°(c 0.626, $CHCl_3$).

(5R)-3-Benzyloxy-5-azidomethyl-Δ^2-isoxazoline (-)-12c: $[\alpha]^{20}_D$ -167.76°(c 0.761, $CHCl_3$).

(5S)-3-Benzyloxy-5-aminomethyl-Δ^2-isoxazoline (+)-12d: $[\alpha]^{20}_D$ +67.49°(c 0.526, $CHCl_3$).

(5R)-3-Benzyloxy-5-aminomethyl-Δ^2-isoxazoline (-)-12d: $[\alpha]^{20}_D$ -67.27°(c 0.605, $CHCl_3$).

(5S)-5-Aminomethyl-3-oxo-isoxazolidine hydrochloride (+)-1.HCl: mp 167-171°C from DMF-acetonitrile; $[\alpha]^{25}_D$ +101.13°(c 0.732, H_2O) [lit.[3] mp 166-172°C; $[\alpha]^{25}_D$ +102°].

(5R)-5-Aminomethyl-3-oxo-isoxazolidine hydrochloride (-)-1.HCl: $[\alpha]^{25}_D$ -101.12°(c 0.683, H_2O) [lit.[3] $[\alpha]^{25}_D$ -103°].

(5S)-5-N(*tert.*Butyloxycarbonyl)aminomethyl-3-oxo-isoxazolidine (+)-3: mp 141.5-142.5°C from 4:1 n.hexane-ethyl acetate; $[\alpha]^{25}_D$ +36.23°(c 1.02, MeOH) [lit.[3] mp 142.0-142.5°C, $[\alpha]^{25}_D$ +37.1°].

(5R)-5-N(*tert.*Butyloxycarbonyl)aminomethyl-3-oxo-isoxazolidine (-)-3: $[\alpha]^{25}_D$ -35.85°(c 1.07, MeOH) [lit.[3] $[\alpha]^{25}_D$ -36.5°].

Acknowledgment. We thank Prof. R.Stradi and Dr. L.Zetta (Milan) for high field NMR spectra, Prof. R.Gandolfi (Pavia) and Prof. P.A.Wade (Philadelphia) for helpful suggestions, Ministero della Pubblica Istruzione (Rome) and NATO (Brussels) for financial support.

References and Notes

1) Curtis, D.R.;Johnston, G.A. *Ergebn.Physiol.* **1974**, *69*, 97.

2) Krogsgaard-Larsen,P.; Hjieds,H.; Curtis, D.R.; Lodge, D; Johnston, G.A.; *J.Neurochem.*, **1979**, *32*, 1717.

3) Krogsgaard-Larsen, P.; Nielsen, L.; Falch, E.; Curtis, D.R. *J.Med.Chem.*, **1985**, *28*, 1612.

4) De Micheli, C.; De Amici M.; Scalfi, M. *Il Farmaco Ed.Sci.*, **1984**, *39*, 487.

5) De Amici, M.; De Micheli, C.; Rodi, R.; Grana, E.; Zonta F.; Santagostino Barbone, M.G. *Eur.J.Med. Chem.*, **1989**, *24*, 171.

6) Caldirola, P.; Ciancaglione, M.; De Amici M.; De Micheli, C. *Tetrahedron Letters*, **1986**, 4647.

7) De Micheli, C.;De Amici, M.; Santagostino Barbone, M.G.;Zonta F.; Grana, E. *Il Farmaco Ed.Sci.*, **1983**, *38*, 817.

8) Caldirola, P.; De Amici M.; De Micheli, C. *Tetrahedron Letters*, **1986**, 4651.

9) Krogsgaard-Larsen, P.; Nordahl-Larsen A.L.; Thyssen, K. *Acta Chem.Scand.,Ser.B*, **1978**, *B32*, 469.

10)Brehm, L.; Jacobsen, P.; Johansen J.S.; Krogsgaard-Larsen, P. *J.Chem. Soc.,Perkin Trans.1*, **1983**, 1459.

11)Castelhano, A.L.; Billedeau, R.; Pliura, D.H.; Bonaventura B.J.; Krantz, A. *Bioorg.Chem.*, **1988**, *16*, 335.

12)Wade, P.A.; Pillay M.K.; Singh, S.M. *Tetrahedron Letters*, **1982**, 4563.

13)De Micheli C.; De Amici, M. *unpublished results.*

14)Chiarino, D.; Napoletano M.; Sala, A. *J.Heterocyclic Chem.*, **1987**, *24*, 43.

15)Grünanger, P.; Vita-Finzi, P. - *Isoxazoles, Part I* - Wiley-Interscience, N.Y., in press.

16)Olive, J.-L.; Petrus, C.; Petrus, F. *Bull.Soc.Chim. France*, **1973**, 1144.

17)A 3-oxo-isoxazolidine was recently prepared by reacting a DMSO solution of a 3-chloroisoxazoline with sodium hydroxide.[11]

18)Crawford, R.J.; Lutener S.B.; Cockcroft, R.D. *Can.J.Chem.*, **1976**, *54*, 3364.

19)In a previous account[6] we reported the erroneous ratio of 86/14. The cycloaddition of (S)-(+)-isopropylidene-3-buten-1,2-diol with three other nitrile oxides gave the following *anti/syn* ratios: [CH_3CNO] 81/19; [C_6H_5CNO] 77/23; [EtOCOCNO] 72/28.

All the nitrile oxides were generated "in situ" from the oxime by addition of sodium hypochlorite (acetonitrile oxide and benzonitrile oxide) or from the hydroximic acid halogenide by addition of solid $NaHCO_3$(bromonitrile oxide and ethoxycarbonyl formonitrile oxide).

20)(a) Kozikowski, A.P.; Ghosh, A.K. *J.Org.Chem.* **1984**, *49*, 2762.

(b) Houk, K.N.; Moses, S.R.; Wu, Y.-D.; Rondan, N.G.; Jäger, V.; Schohe, R.; Fronczek, F.R. *J.Am.-Chem.Soc.*, **1984**, *106*, 3880.

(c) Wade, P.A.; Singh, S.M.; Pillay, M.K. *Tetrahedron*, **1984**, *40*, 601.

(d) Houk, K.N.; Duh, H.-Y.; Wu, Y.-D.; Moses, S.R. *J.Am.Chem.Soc.* **1986**, *108*, 2754.

(e) Curran, D.P.; Kim, B.H. *Synthesis*, **1986**, 312.

21) Jäger, V.; Schohe, R. *Tetrahedron*, **1984**, *40*, 2199.

22) Vyas, D.M.; Chiang Y.; Doyle, T.W. *Tetrahedron Letters*, **1984**, 487.

23) Dale, J.A.; Dull D.L.; Mosher, H.S. *J.Org.Chem.*, **1969**, *34*, 2543.