# Indole Grignard Reaction. III.[1] Synthesis, Crystal Structure, and Analgesic Activity of (*R*)- and (*S*)-3-Amino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indoles

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**Both enantiomers of the title compounds were synthesized and the crystal structure of one of them was determined by X-ray crystallography. The indole Grignard reaction was effective for synthesizing the key intermediate of the (*R*)-isomers. The analgesic activities of the products were compared with those of 4-methylaminomethyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (1), Isoxal®, and Tiaramide®. The (*R*)-isomers were more potent than the corresponding (*S*)-isomers, and were more potent than Isoxal and Tiaramide, but less potent than 1..**

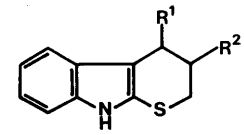
**Keywords** thiopyrano[2,3-*b*]indole; indole Grignard reaction; analgesic activity; X-ray crystallography; enantiomer

We have reported the structure–analgesic activity relationships, methods of synthesis, and conformational studies of 4-methylaminomethyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indoles (**1**).[1a-c] Recently, based on analyses of the three-dimensional structure of **1**, we proposed a model of the interaction between **1** and the putative active site of the receptor through edge-to-face aromatic–aromatic interaction *via* formation of the pseudo cyclic substructure.[1b]

Molecular modeling studies suggested that compound **2** may form a similar pseudo cyclic substructure, though less favorably than **1**, during conformational interconversion. In order to obtain further insights into the structure–activity relationships, we prepared both enantiomers of **2**, **3**, and **4** and determined the crystal structure of **2** by X-ray crystallography. We also report a brief comparison of the analgesic activities of **2**—**4** and **1**,[1c] Isoxal® and Tiaramide® using some animal models.

**Syntheses and X-Ray Crystallography** L-Tryptophan (L-Trp) was converted to the thiol (**8**) in 52% overall yield. Cyclization of **8** to the desired tricyclic system (**9**) was conducted at −78 to 0 °C by a modification of the method of Hino *et al.*[2] using *N*-bromosuccinimide (NBS, 0.98 eq) in methylene chloride and propylene oxide (2 : 1), in 79% yield. Reductive cleavage of the *p*-toluenesulfonyl (tosyl) group of **9** with sodium and naphthalene in dimethoxyethane gave (*S*)-**2** (77%).

Regiospecific introduction of the C-terminal of L-cysteine



**1**: $R^1=CH_2NHMe$, $R^2=H$
**2**: $R^1=H$, $R^2=NH_2$
**3**: $R^1=H$, $R^2=NHMe$
**4**: $R^1=H$, $R^2=N(Me)_2$

Fig. 1

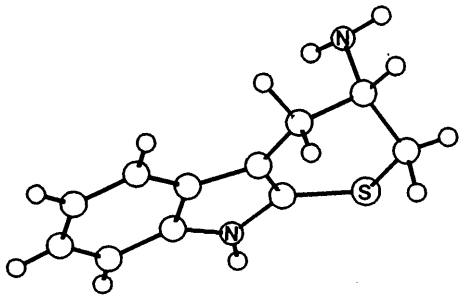
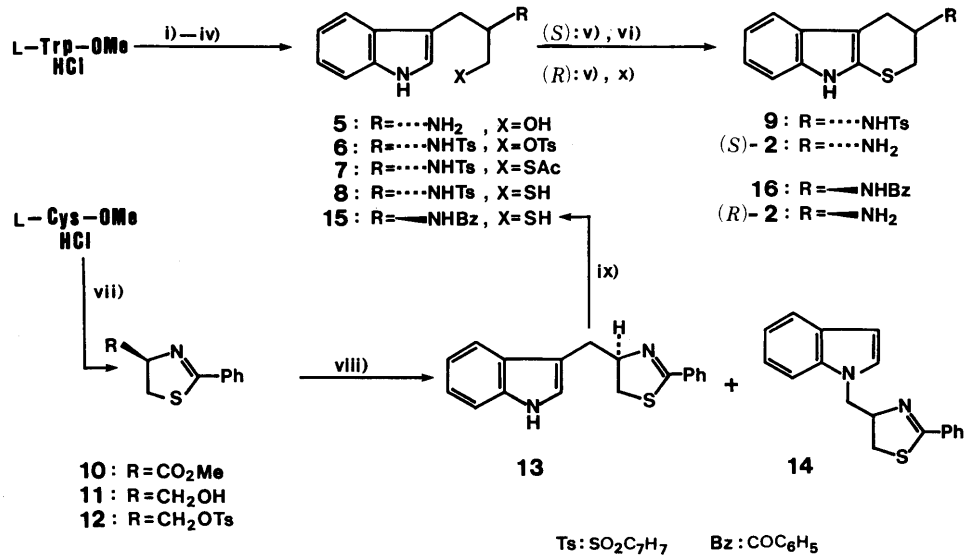


Fig. 2




reagents; i) $NaBH_4$, ii) TsCl, iii) $CH_3COSK$, iv) KOH, v) NBS/$CH_2Cl_2$–propylene oxide, vi) Na, naphthalene, vii) PhC(=NH)OEt, viii) EtMgBr, ix) HCl–KCl, x) HCl

Chart 1

TABLE I

| No. | Methods[a] | | |
|---|---|---|---|
| | AcOH writhing | PQ writhing | Foot licking |
| (*S*)-**2** | >50 | 150 | >25 |
| (*R*)-**2** | 27.4 | 175 | >25 |
| (*S*)-**3** | 23.2 | 175 | 25 |
| (*R*)-**3** | 3.2 | 75 | 8.6 |
| (*S*)-**4** | 21.9 | 150 | >25 |
| (*R*)-**4** | 14.0 | 35 | >25 |
| (±)-**1**[b] | 4.2 | 5.6 | 2.0 |
| Isoxal | 63 | 157 | 23 |
| Tiaramide | 55 | 144 | 150 |

*a*) AcOH, acetic acid; PQ, phenyl quinone. $ED_{50}$ (mg/kg, *p.o.*). *b*) Data are taken from Ref. 1*c*.

(L-Cys) into the C-3 position of indole followed by cyclization at C-2 with the sulfur moiety may be able to afford (*R*)-**2**, for we have found that the reactions of indolylmagnesium halides and alkylating reagents proceed effectively when the reactions are carried out in benzene with the sulfonate ester as the leaving group.[1a] Therefore, we applied our method to this case, and L-Cys was converted to the thiazoline **12** in 61% yield.[3] The reaction of indolylmagnesium bromide [prepared from indole (1.0 eq) and ethylmagnesium bromide (1.1 eq)] and **12** (1.5 eq) at 10 to 25 °C in benzene afforded an excellent yield of the C3-substituted indole **13** (91%) and a small amount of the regioisomer **14** (6%).[4] Compound **13** was carefully hydrolyzed to give the benzoylaminothiol (**15**), which was cyclized with NBS as described above, giving **16** in 86% yield.[2] Acid hydrolysis of **16** afforded (*R*)-**2** (75%). The crystal structure of **2** is shown in Fig. 2.[5]

Both (*S*)- and (*R*)-**2** were converted to the corresponding mono- and dimethylamino derivatives, **3** and **4**.

**Analgesic Activity** Analgesic activities were compared after oral administration by means of the mouse acetic acid writhing,[6] phenylquinone writhing,[7] and foot licking methods.[8] The results are summarized in Table I. The data for **1**,[1c] Isoxal, and Tiaramide are also listed for comparison. The (*R*)-enantiomers of **2**—**4** are more potent than the corresponding (*S*)-isomers, as in the case of **1**.[1b] Compounds **2**—**4** exert much more potent analgesic activity than Isoxal and Tiaramide.

## Experimental

Melting points are uncorrected. Proton and carbon-13 nuclear magnetic resonance ($^1$H and $^{13}$C-NMR) spectra were recorded on Varian EM-360 (60 MHz) and XL-100-12A (25.16 MHz) instruments with tetramethylsilane as the internal standard, respectively. Infrared spectra (IR) were recorded on a Hitachi 260-10 spectrometer. Optical rotations were measured at 23 °C in a 10-cm cell on a Perkin Elmer 141 polarimeter. Unless otherwise stated, organic extracts were washed with saturated NaCl and dried over $MgSO_4$. Details of the pharmacological experiments have been described elsewhere.[1] X-Ray measurements were performed on a Rigaku AFC-5 diffractometer. A total of 1020 unique reflections were recorded (Cu $K_\alpha$ radiation; $\lambda = 1.54178$Å) in the range $\theta \leqq 65°$; 1005 reflections with $I \geqq 2\sigma(I)$ were considered as observed. The structure was solved by direct methods. All of the hydrogens could be identified in a difference density map and were included in the refinement with isotropic temperature factors. The structure was refined by the block-diagonal least squares method with anisotropic temperature factors for the non-hydrogen atoms. Final residuals were $R$ 0.035 and $R_w$ 0.037.[5]

**β-(*S*)-*p*-Toluenesulfonylamino-3-indolepropanethiol (8)** **5**[9] (32.7 g, 0.17 mol) and tosyl chloride (68.2 g, 0.36 mol) were stirred at 3 °C in pyridine (180 ml) for 14 h. The mixture was poured onto ice and acidified with HCl. The layers were extracted with EtOAc to obtain **6** (oil, 79.6 g). Unpurified **6** was reacted with potassium thioacetate (39 g, 0.34 mol) in dimethylformamide (DMF) (1 l) at 65—70 °C for 2 h. The solvent was removed and the residue was extracted with ether. **7**: colorless prisms (50 g, 73% from **5**).

**7** (50 g, 124 mmol) was hydrolyzed with KOH (7.0 g, 125 mmol) in refluxing 20% aqueous EtOH (1 l) for 1 h. The solvent was removed and the aqueous layer was acidified with concentrated HCl, and extracted with ether. **8**: colorless prisms (40.7 g, 52% overall yield from L-Trp).

**(*S*)-3-Tosylamino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (9)** A solution of NBS (2.4 g, 135 mmol) in $CH_2Cl_2$ (250 ml) was added at −78 °C to a stirred solution of **8** (5.0 g, 138 mmol) in $CH_2Cl_2$ (150 ml) and propylene oxide (75 ml) over 3 h. After 2 h, the reaction mixture was allowed to warm to 0 °C over a 2 h period. The mixture was diluted with tetrahydrofuran (THF), and washed with 10% NaOH and water. **9**: colorless prisms (3.98 g, 79%): $^{13}$C-NMR (pyridine-$d_5$) $\delta$: 21.2 ($CH_3$), 28.3 (C4), 33.2 (C2), 48.9 (C3), 105.2 (C4a), 110.6 (C8), 116.4 (C5), 119.4 (C6), 121.0 (C7), 125.9 (C4b), 129.1 (C9a), 137.4 (C8a), 127.1, 130.0, 139.8, 143.1 (Ts).

**(*S*)-3-Amino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (2)** Na (900 mg, 0.039 atom) was added at 25 °C to a solution of naphthalene (5.0 g, 39 mmol) in DME (16 ml). After 2 h, a solution of **9** (3.0 g, 8.4 mmol) in DME (45 ml) was added dropwise. The reaction was continued for 2 h, and quenched by adding water. The mixture was extracted with cold HCl. The acid layers were washed with EtOAc, then NaOH pellets were added. The precipitated **2** was collected: colorless needles (1.30 g, 76%): $^{13}$C-NMR (pyridine-$d_5$) $\delta$: 30.9 (C4), 36.0 (C2), 47.0 (C3), 106.4 (C4a), 110.7 (C8), 116.6 (C5), 119.4 (C6), 120.8 (C7), 126.1 (C4b), 129.7 (C9a), 137.8 (C8a).

**(*S*)-3-Methylamino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (3)** Ethyl chloroformate (983 mg, 9.14 mmol) was added at 23 °C to a stirred solution of **2** (1.77 g, 8.70 mmol) and $Et_3N$ (2.43 ml) in THF (85 ml). After 1 h, the volatiles were removed and the residue was extracted with EtOAc. The organic layer was washed with 3 M HCl and brine, dried and concentrated. The residue was directly treated with $LiAlH_4$ (1.31 g, 34 mmol) in refluxing THF (50 ml) for 2 h to obtain (*S*)-**3**: colorless needles (1.74 g, 92%).

**(*S*)-3-Dimethylamino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (4)** Aqueous formaldehyde (37%, 6.3 ml), AcOH (1.9 ml), and $NaBH_3CN$ (1.17 g) were added to a solution of (*S*)-**2** (1.51 g, 7.4 mmol) in MeOH (40 ml)–THF (40 ml). After 1 h, the solvent was removed, and 10% NaOH was added to the residue. The layers were extracted with EtOAc. (*S*)-**4**: colorless solid (1.32 g, 77%).

**2-Phenyl-4-tosyloxymethyl-2-thiazoline (12)** L-Cys-OMe·HCl (117 g, 0.685 mol) and ethylbenzimidate (92.2 g, 0.620 mol) were stirred in MeOH (520 ml) at 24 °C for 90 min.[3] The solvent was removed below 10 °C and the residue was extracted with cold benzene. Recrystallization from MeOH was carried out below 5 °C throughout in order to avoid racemization. **10**: colorless prisms (117.6 g, 86%).

**10** (22.1 g, 0.100 mol) was reduced with $LiAlH_4$ (4.5 g) in ether (250 ml) at 3 °C for 30 min to obtain **11** (colorless needles, 14.8 g, 77%), which was treated with tosyl chloride (14.8 g, 78 mmol) in pyridine (148 ml) at 5 °C for 19 h. **12**: colorless prisms (24.6 g, 92%).

**The Reaction of Indole and 12** A solution of indole (5.0 g, 43 mmol) in benzene (15 ml) was cooled to 10 °C, then a 3.1 M ether solution of EtMgBr (15.0 ml, 46.5 mmol) was added, followed by **12** (10.0 g, 28.8 mmol) in benzene (100 ml). The mixture was stirred at 10 °C for 2.5 h and 25 °C for 3 h, and quenched by adding aqueous $NH_4Cl$. The layers were extracted with ether. Purification of the products on silica gel (300 g, EtOAc–benzene = 1 : 99) afforded the following products in the order of elution: 1) Indole (1.9 g), 2) **14**; oil (505 mg, 6%), 3) **13**; colorless crystals (7.65 g, 91%).

**Acid Hydrolysis of 13** A mixture of **13** (5.0 g, 17.1 mmol), 0.2 M HCl (90 ml, 18.0 mmol), 0.1 M KCl (80 ml), and MeOH (40 ml) was refluxed for 20 h. MeOH was removed and the aqueous layer was extracted with $CHCl_3$. The $CHCl_3$ layer was extracted with 1 M KOH. The aqueous KOH phase was acidified with concentrated HCl, then extracted with EtOAc. **15**: colorless crystals (3.5 g, 62%).

**(*R*)-3-Benzoylamino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (16)** By a method similar to that described for the cyclization of **8** to **9**, **15** (1.90 g, 6.13 mmol) was converted to **16**: colorless crystals (1.63 g, 86%).

**(*R*)-3-Amino-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (2)** **15** (4.40 g, 14.3 mmol) was refluxed in DME (150 ml) and concentrated HCl (150 ml) for 17 d. The mixture was concentrated under reduced pressure. The

TABLE II. $^1$H-NMR and IR Data

| No. | $^1$H-NMR ($\delta$ ppm) (Solvent) | IR ($\nu_{max}$ cm$^{-1}$) (Solvent) |
|---|---|---|
| **2** | (Pyridine-$d_5$) 2.65—3.3 (4H, m, $CH_2\times2$), 3.63 (1H, m, CH), 6.9—7.5 (4H, m, Ar), 8.35 (1H, br s, NH) | |
| **3** | ($CDCl_3$) 2.43 (3H, s, $CH_3$), 2.8—3.3 (5H, m, $CH_2CHCH_2$), 6.9—7.6 (4H, m, Ar) | |
| **4** | (Pyridine-$d_5$) 2.32 (6H, s, $(CH_3)_2$), 3.0—3.3 (5H, m, $CH_2CHCH_2$), 7.2—7.7 (4H, m, Ar) | |
| **7** | (Acetone-$d_6$) 2.23 (3H, s, $CH_3$), 2.32 (3H, s, $COCH_3$), 2.85—3.12 (4H, m, $CH_2\times2$), 3.65 (1H, m, CH), 6.88—7.60 (9H, m, Ar) | 3470 (NH), 1690 (CO) ($CHCl_3$) |
| **8** | ($CDCl_3$) 2.32 (3H, s, $CH_3$), 2.54—2.97 (4H, m, $CH_2\times2$), 3.50 (1H, m, CH), 4.92 (1H, d, $J$=6.5 Hz, NH), 7.0—7.6 (9H, m, Ar) | 3470 (NH), 2580 (SH) ($CHCl_3$) |
| **9** | (Acetone-$d_6$) 2.40 (3H, s, $CH_3$), 2.7—3.2 (5H, m, $CH_2CHCH_2$), 3.93 (1H, br s, NH), 6.8—7.83 (8H, m, Ar) | |
| **12** | ($CDCl_3$) 2.43 (3H, s, $CH_3$), 3.1—3.7 (2H, m, $SCH_2$), 3.95—4.5 (2H, m, $OCH_2$), 4.95 (1H, m, CH), 7.2—7.9 (5H, m, Ar) | |
| **13** | (Acetone-$d_6$) 2.9—3.6 (4H, m, $CH_2\times2$), 5.00 (1H, m, CH), 6.9—7.6 (10H, m, Ar) | 3480 (NH) ($CHCl_3$) |
| **14** | ($CDCl_3$) 2.9—3.4 (2H, m, $SCH_2$), 4.0—4.7 (2H, m, $NCH_2$), 5.11 (1H, m, CH), 6.52 (1H, d, $J$=2 Hz, $\beta$-H), 6.9—7.5 (10H, m, Ar) | |
| **15** | ($CDCl_3$) 1.37 (1H, t, $J$=8 Hz, SH), 2.73 (2H, dd, $J$=7 and 8 Hz, $SCH_2$), 3.2 (2H, m, $CH_2$), 4.6 (1H, m, CH), 6.58 (1H, br s, NH), 6.9—7.7 (10H, m, Ar) | 3480 (NH), 1660 (CO) ($CHCl_3$) |
| **16** | (Pyridine-$d_5$) 3.43 (2H, d, $J$=5 Hz, $SCH_2$), 3.10, 3.28 (2H, m, $J$=16, 7 and 5 Hz, $CH_2$), 5.15 (1H, m, CH), 6.95—7.5 (9H, m, Ar) | 1625 (CO) (Nujol) |

TABLE III. Melting Points, $[\alpha]_D$, Elemental Analysis and MS Data

| No. | mp (°C) (Recryst. solvent[a]) | $[\alpha]_D$ 23 °C ($c$, solvent) | Formula | Calcd (C, H, N, S) (Found) | | | | MS $m/z$ ($M^+$) |
|---|---|---|---|---|---|---|---|---|
| **7** | 134.0—136.0 (MeOH) | −31.6 (1.971, EtOH) | $C_{20}H_{22}N_2O_3S_2$ | 59.68 (59.71 | 5.51 5.70 | 6.96 6.83 | 15.93 15.92) | 402 |
| **8** | 128.0—130.0 (MeOH) | −79.5 (2.065, EtOH) | $C_{18}H_{20}N_2O_2S_2$ | 59.97 (59.98 | 5.59 5.55 | 7.77 7.68 | 17.79 17.59) | 360 |
| **9** | 222 (dec.) (MeOH) | +86.2 (1.066, acetone) | $C_{18}H_{18}N_2O_2S_2$ | 60.31 (60.02 | 5.06 5.01 | 7.81 7.48 | 17.89 17.69) | 358 |
| **10** | 42.5—43.5 (MeOH) | +59.7 (2.374, $CHCl_3$) | $C_{11}H_{11}NO_2S$ | 59.71 (59.69 | 5.01 4.98 | 6.33 6.24 | 14.49 14.53) | |
| **11** | 65.5—66.5[b] (Acetone–PE) | +108.0[b] (1.712, $CHCl_3$) | $C_{10}H_{11}NOS$ | 62.15 (62.34 | 5.73 5.76 | 7.25 7.25 | 16.59 16.59) | |
| **12** | 85.5—86.5 (MeOH) | +84.5 (2.126, $CHCl_3$) | $C_{17}H_{17}NO_3S_2$ | 58.77 (58.68 | 4.93 4.89 | 4.03 4.10 | 18.46 18.36) | |
| **13** | 140.0—141.0 (MeOH) | +10.1 (1.017, acetone) | $C_{18}H_{16}N_2S$ | 73.94 (74.09 | 5.51 5.53 | 9.58 9.69 | 10.97 11.11) | 292 |
| **15** | 137.0—139.0 (MeOH) | +29.7 (1.064, MeOH) | $C_{18}H_{18}N_2OS$ | 69.65 (69.69 | 5.84 5.89 | 9.02 9.00 | 10.33 10.40) | |
| **16** | 252—259 (dec.) (Acetone) | +221.5 (1.269, dioxane) | $C_{18}H_{16}N_2OS$ | 70.10 (70.14 | 5.23 5.24 | 9.08 8.91 | 10.40 10.66) | 308 |
| (*S*)-**2** | 232 (dec.) (MeOH) | +70.9 (0.788, DMSO) | $C_{11}H_{12}N_2S$ | 64.67 (64.77 | 5.92 5.96 | 13.71 13.64 | 15.69 15.57) | 204 |
| (*R*)-**2** | 231 (dec.) (MeOH) | −69.5 (0.722, DMSO) | $C_{11}H_{12}N_2S$ | 64.67 (64.73 | 5.92 5.92 | 13.71 13.50 | 15.69 15.48) | |
| (*S*)-**3** | 207.0—209.0 (EtOH) | +89.0 (1.204, DMSO) | $C_{12}H_{14}N_2S$ | 66.02 (66.11 | 6.46 6.39 | 12.83 12.77 | 14.69 14.59) | |
| (*R*)-**3** | 206.0—208.0 (EtOH) | −86.9 (0.741, DMSO) | $C_{12}H_{14}N_2S$ | 66.02 (65.86 | 6.46 6.41 | 12.83 12.63 | 14.69 14.91) | |
| (*S*)-**4** | 197.5—198.5 (EA) | +129.2 (0.984, MeOH) | $C_{13}H_{16}N_2S$ | 67.20 (67.21 | 6.94 6.95 | 12.06 12.00 | 13.80 13.70) | |
| (*R*)-**4** | 196.5—198.0 (MeOH) | −126.9 (0.954, MeOH) | $C_{13}H_{16}N_2S$ | 67.20 (67.05 | 6.94 6.94 | 12.06 11.90 | 13.80 13.61) | |
| i | 105.0—107.0 (EA–$Et_2O$) | −162.2 (1.012, $CHCl_3$) | $C_{18}H_{21}NO_5S_3$ | 50.57 (50.63 | 4.95 4.97 | 3.28 3.22 | 22.50 22.40) | |
| ii | 73.0—75.0 (EtOH) | +49.7 (0.988, $CHCl_3$) | $C_{19}H_{21}NO_5S_2$ | 56.00 (56.00 | 5.19 5.19 | 3.44 3.43 | 15.74 15.72) | |

*a*) EA, ethyl acetate; PE, petroleum ether (bp 35—60 °C). *b*) Lit.[3] mp 75—76 °C, $[\alpha]_D$ 0° (EtOH).

starting material was recovered by filtration (1.29 g, 29%). The aqueous layer was treated with NaOH to obtain **2**: colorless prisms (1.50 g, 51%).

(*R*)-**2** was converted to (*R*)-**3** and (*R*)-**4** by the same methods as described for the (*S*)-isomers.

**Crystal Data of 2** $C_{11}H_{12}N_2S$ (204.3), orthorhombic, $P2_12_12_1$, $a$=11.531(1), $b$=13.070(1), $c$=6.712(1) Å, $V$=1011.4(1) Å$^3$, $Z$=4, $D_c$=1.341 g/cm$^3$, $\mu$(Cu $K_\alpha$)=2.42 mm$^{-1}$, $F$(000)=432.

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**References and Notes**

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4) Similarly, thiazolidines i or ii (Fig. 3) could afford the corresponding C3-substituted indoles in 79% and 76% yields, respectively. However, the iodide (**12**, $R=CH_2I$) gave **13** in 14% yield after refluxing in benzene for 17 h.

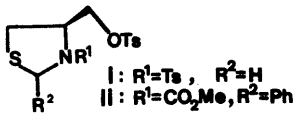


Fig. 3

5) Atomic coordinates, and bond and thermal parameters of **2** have been deposited at the Cambridge Crystallographic Data Centre.
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