(*R*)- and (*S*)-4-Methylaminomethyl-2,3,4,9-Tetrahydrothiopyrano[2,3-*b*]indole: Synthesis,† Absolute Configuration, Conformation, and Analgesic Activity

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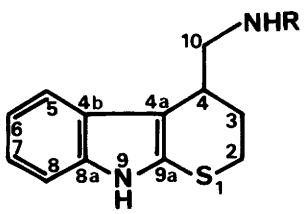
(*R*)- and (*S*)-4-Methylaminomethyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (**2**) have been synthesized and their conformation studied. (*RS*)-(**2**) Has been found to be a potent non-narcotic analgesic agent. The crucial step was regio- and stereo-specific cyclization of indolyl alkyl sulphides (*S*)- and (*R*)-(**9**) to both enantiomers of (**10**) using an intramolecular indole Grignard reaction. Optical purities and the absolute configuration were established. The analgesic activity of (*R*)-(**2**) is more potent than that of the (*S*)-isomer. The thiopyrano ring moiety of (**2**) was shown to adopt a half-chair conformation with pseudoaxial orientation of the side chain by X-ray and NMR methods. In the crystal structure of the *p*-bromobenzylamino derivative of (**2**), two six-membered aromatic rings were found to adopt a perpendicular edge-to-face arrangement, which has led us to propose a model for drug-receptor binding. The roles of configuration and conformation as they affect the analgesic activity are discussed.

We have disclosed the structure–activity relationships of a novel analgesic compound, 4-aminomethyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole (**1**) and its 42 analogues.[1] Among them, (**2**) was found to display excellent activity in a wide range of animal models predictive of antinociceptive activity including modified Haffner, foot-licking, and Randall Selitto methods following oral administration. Compound (**2**) exhibited almost equivalent potency to morphine but was not a narcotic agonist or antagonist.[1] Furthermore, (**2**) was not structurally related to any other potent analgesic drugs. These features led to the characterization of (**2**) as a novel class of analgesics, and prompted us to examine the stereochemistry and activity relationships.



(**1**) R=H
(**2**) R=Me

Figure 1.

In the preceding paper, we described both a novel method for constructing the 2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indole ring system using an intramolecular indole Grignard reaction together with a conformational study by means of NMR spectroscopy.[2] Here we report on the syntheses of both enantiomers of (**2**), describe the determination of their optical purities and absolute configurations, and briefly give the results of a preliminary study of their analgesic activity. The conformational properties of (**2**) were studied by means of X-ray and NMR methods. Finally, the effect of stereochemical contributions on activity are discussed.

Results

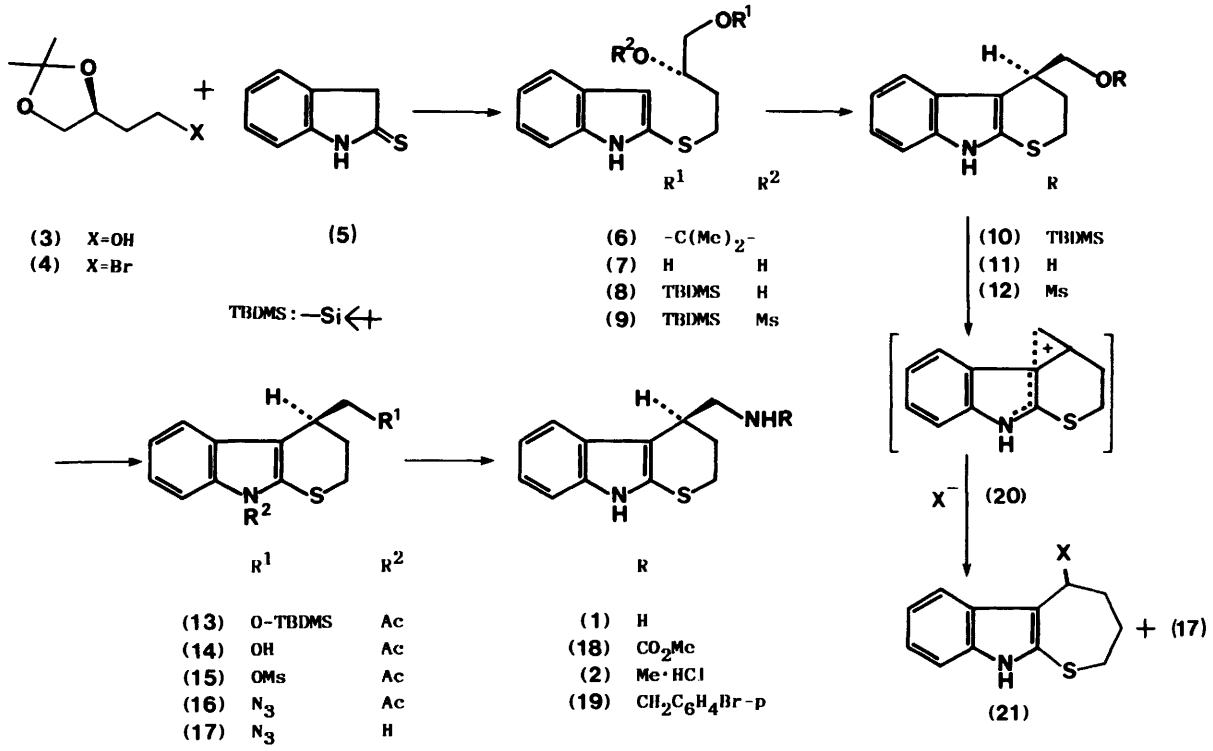
Synthesis of (*S*)-(**2**).—Compound (*S*)-(**2**) was synthesized as shown in Scheme 1. The chiral starting material, the acetonide alcohol (**3**), readily obtainable from (*S*)-malic acid by the method of Mori *et al.*,[3] was conventionally converted into the bromide (*S*)-(**4**). Condensation of (**4**) with the indole-2-thione (**5**)[1] gave the indolyl sulphide (**6**). The acetonide group of (**6**) was removed by treatment with aqueous HCl to yield the diol (**7**). Selective protection of the terminal hydroxy group of (**7**) with t-butyldimethylchlorosilane in the presence of a catalytic quantity of 4-*N*,*N*′-dimethylaminopyridine (DMAP)[4] afforded the (*S*)-alcohol (**8**), $[\alpha]_D^{23}$ + 16.8 ($CHCl_3$), in 80% overall yield from (**5**).

Tetrahydrothiopyrano[2,3-*b*]indole‡ ring formation, the crucial step in these syntheses, was achieved *via* an intramolecular indole Grignard reaction.[2] The alcohol (**8**) was quantitatively mesylated with methanesulphonic anhydride (MSA) and triethylamine in methylene dichloride at −13 °C to give (**9**) which, without purification, was treated with ethylmagnesium bromide (EtMgBr) in benzene at 10 °C for 10 min to afford the silyl ether (**10**) regiospecifically in good yield. Alternatively, (**10**) could be easily obtained by mesylation of (**8**) with MSA and triethylamine in methylene dichloride, followed by *in situ* treatment with dropwise addition of EtMgBr at −13 °C. Without purification, (**10**) was deprotected to yield the optically active alcohol (**11**), $[\alpha]_D^{23}$ + 55.0 ($CHCl_3$), in 81% yield from (**8**). The spectral properties of (**11**) were identical with those of the racemate.[2]

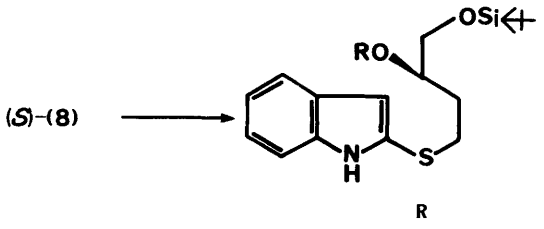
Unpurified silyl ether (**10**) was used as the starting material for a conversion of the hydroxy group into the required amine function of (**2**). Acetylation of the indole nitrogen of (**10**) at 23 °C with acetic anhydride and pyridine in the presence of a catalytic quantity of DMAP, followed by desilylation with aqueous HF in acetonitrile yielded the *N*-protected alcohol (**14**) in 63% overall yield from (**8**). Reaction of (**14**) with MSA afforded the mesylate (**15**), which on treatment with sodium azide in DMF at 50 °C for 16 h, followed by alkaline hydrolysis of the *N*-acetyl group gave the pure azide (**17**), $[\alpha]_D^{23}$ + 68.4 ($CHCl_3$), in 77% yield after recrystallization from methanol. Protection of the indole nitrogen with an electron-withdrawing

† This work was presented in part at the 9th International Congress of Heterocyclic Chemistry, Tokyo, Japan, Aug. 21–26, 1983. See: Abstracts of Papers, 1983, p. 397.

‡ The descriptors 2,3,4,9- of tetrahydrothiopyrano[2,3-*b*]indoles are omitted hereafter.



Scheme 1.



Scheme 2.

functional group was necessary, since the direct replacement of (**12**) with various nucleophiles, sodium azide for example, under a variety of reaction conditions yielded a considerable amount of ring-expanded compound (**21**), presumably *via* the cyclopropylium cation (**20**). The acetyl and methoxycarbonyl groups (R^2 = Ac or CO_2Me) were equally effective, and the yield of (**21**) could be almost completely suppressed (HPLC analysis). Unwanted (**21**; X = N_3) was readily removed by recrystallization.

Synthesis of the optical active amine (*S*)-(**1**), m.p. 139–140 °C, $[\alpha]_D^{23}$ + 77.6 (MeOH), was accomplished by reduction of (**17**) with $LiAlH_4$ in ether at 23 °C in 95% yield. Its spectral properties were identical with those of racemic (**1**).[1] Treatment of (**1**) with methyl chloroformate afforded the urethane (**18**), which was reduced by $LiAlH_4$ in refluxing THF to give (*S*)-(**2**) as an oil. Exposure of (**2**) to a solution of anhydrous HCl in ethanol afforded the HCl salt, m.p. 265–272 °C (decomp), $[\alpha]_D^{23}$ + 68.6 (MeOH), in 79% yield from (**1**). The total overall yield of (**2**) was 5.9% over 16 steps from (*S*)-malic acid.

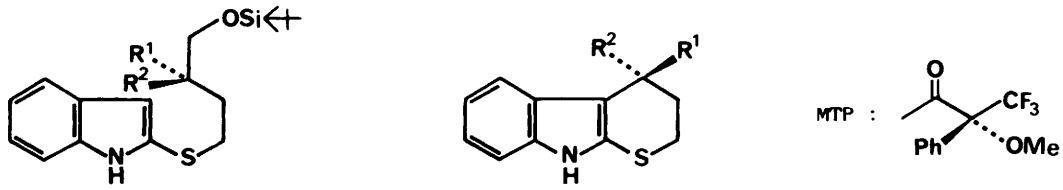
Synthesis of (*R*)-**2**.—The starting material for the synthesis of (*R*)-(**2**) is (*S*)-(**8**), an intermediate used for the synthesis of the corresponding (*S*)-enantiomer. Inversion of the configuration at the asymmetric centre of (*S*)-(**8**) could afford antipodal (**8**) which could lead to (*R*)-(**2**). The Mitsunobu reaction is the method of choice for this purpose.[5] However, the primary silyl group of (**8**) was found both to undergo migration and cleavage under a variety of alkaline or reductive reaction conditions to give a mixture of isomeric silyl ethers or diols. After many unsuccessful trials, we obtained (*R*)-(**8**) in excellent chemical and optical yield as illustrated in Scheme 2.

The reaction of (*S*)-(**8**) with acetylsalicylic acid, triphenylphosphine, and diethyl azodicarboxylate in THF at −13 °C for 1 h and then at 3 °C for 30 min afforded the salicylate (**22**), which was cleaved by $LiAlH_4$ in ether at −65 °C. Careful work-up by dropwise addition of water provided (*R*)-(**8**) in 80% yield from (*S*)-(**8**), $[\alpha]_D^{23}$ + 17.2 ($CHCl_3$). The $[\alpha]_D$ value of (+)-(**8**) agreed well with that of (*S*)-(−)-(**8**).

Compound (*R*)-(**8**) was converted into (*R*)-(**2**)·HCl, m.p. 265–272 °C (decomp), $[\alpha]_D^{23}$ − 68.2 (MeOH), by the methods described for the synthesis of (*S*)-(**2**).

Determination of Optical Purities.—Although the physical constants of both (*S*)- and (*R*)-(**2**) suggested that they were enantiomerically homogeneous, further experiments to estimate optical purities were carried out using both enantiomers of the intermediates (**8**), (**11**), and (**1**). The method is based on the reaction of each enantiomer with optically pure carboxylic acid, followed by an analysis of the resulting diastereoisomeric esters or amides by high-performance liquid chromatography (HPLC) (Scheme 3).

Reactions of (*S*)- and (*R*)-(**8**) with (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) in the presence of DMAP and dicyclohexylcarbodi-imide (DCC)[6] resulted in the formation of (*SR*)-(**23**) (93%) and (*RR*)-(**23**) (94%). Normal phase analytical HPLC completely separated two peaks at *ca.* 10.5 min for (*RR*)-(**23**) and 11.3 min for (*SR*)-(**23**), when they had been admixed. Separately, (*SR*)-(**23**) and (*RR*)-(**23**) each gave essentially a single peak (1 : 99 and 99.5:0.5, respectively). Thus, the enantiomeric excesses of the key starting materials (*S*)-(**8**) and (*R*)-(**8**) were *ca.* 98% e.e. and 99% e.e., respectively.



	R^1	R^2
(*SR*)-(23)	O-MTP	H
(*RR*)-(23)	H	O-MTP

	R^1	R^2
(*SR*)-(24)	CH_2O-OMM	H
(*RR*)-(24)	H	CH_2O-OMM
(*SR*)-(25)	CH_2NH-MTP	H
(*RR*)-(25)	H	CH_2NH-MTP

Scheme 3.

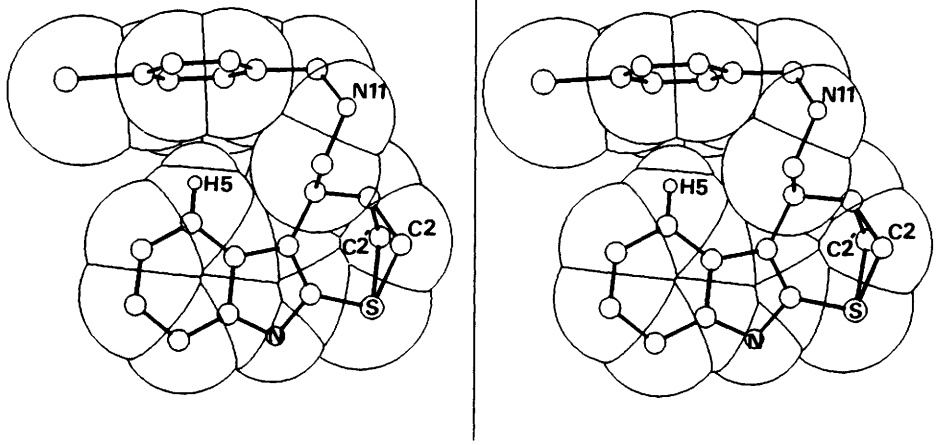


Figure 2. Stereoscopic view of the crystal structure of (+)-(**19**). Hydrogens except for 5-H were removed for clarity. The C(2) atom is disordered at the two positions which are presented by C(2) and C'(2), respectively.

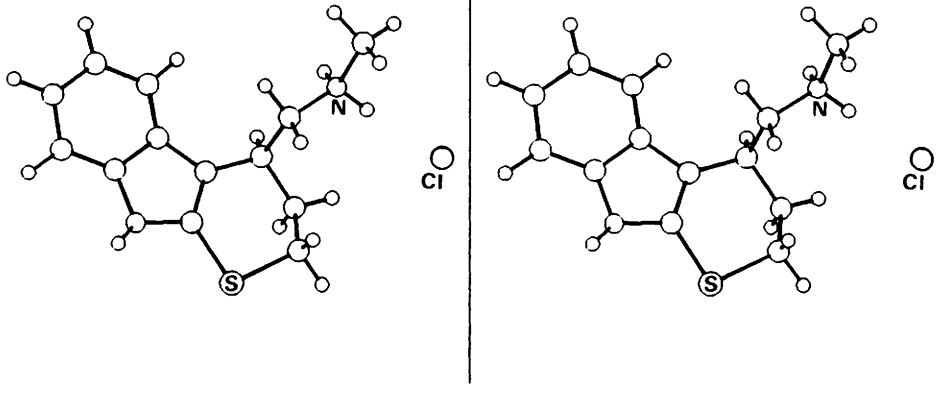


Figure 3. Stereoscopic view of the crystal structure of (**2**)·HCl.

In a similar manner, (*S*)- and (*R*)-(**11**) were converted into their diastereoisomeric esters, (*SR*)-(**24**) and (*RR*)-(**24**), respectively. In this case, (*R*)-*O*-methylmandelic acid[7]* was used instead of MTPA, since the corresponding MTPA esters resulted in insufficient separation. HPLC analysis demonstrated that (*SR*)-(**24**) prepared from (*S*)-(**11**) {$[\alpha]_D^{23}$ + 55.0 ($CHCl_3$)} contained *ca.* 2.5% of the diastereoisomeric isomers (95% e.e.).

Both enantiomers of the amine (**1**) were converted into the amides of (*R*)-MTPA with DMAP–DCC in 98–100% yields.

* (*R*)-(−)*O*-methylmandelic acid was prepared by resolution of racemate *via* an 1-ephedrine salt,[7] m.p. 187–189 °C, $[\alpha]_D^{23}$ − 74.6° (*c.* 1.16, MeOH). Optical purity was considered satisfactory based on comparison with m.p.s and $[\alpha]_D$ value with those reported in ref. 7.

Table 1. Atomic co-ordinates ($\times 10^4$) with esds in parentheses for (**2**)·HCl[a]

	x	*y*	*z*
S(1)	5 102(1)	2 440(4)	7 942(1)
C(2)	6 129(5)	1 817(6)	10 099(5)
C(3)	7 898(4)	2 260(5)	11 011(4)
C(4)	8 128(3)	3 861(5)	11 302(4)
C(4a)	7 214(3)	4 643	9 682(4)
C(4b)	7 426(3)	6 089(5)	9 305(4)
C(5)	8 502(4)	7 149(5)	10 231(4)
C(6)	8 314(5)	8 474(5)	9 465(5)
C(7)	7 116(6)	8 722(6)	7 844(5)
C(8)	6 057(4)	7 659(6)	6 903(5)
C(8a)	6 230(4)	6 363(5)	7 659(4)
N(9)	5 348(3)	5 133(5)	7 038(3)
C(9a)	5 934(4)	4 125(5)	8 299(4)
C(10)	7 605(4)	4 432(5)	12 602(4)
N(11)	8 739(3)	4 045(5)	14 361(3)
C(12)	8 302(5)	4 708(7)	15 643(5)
Cl	8 416(1)	939(4)	15 253(1)

[a] Atom numbers are shown in Figure 1.

Table 2. Bond lengths (Å) and bond angles (°) with esds in parentheses for (**2**)·HCl

C(5)–C(6)	1.402(7)	C(5)–C(4b)	1.396(7)
C(6)–C(7)	1.391(7)	C(7)–C(8)	1.395(8)
C(8)–C(8a)	1.375(7)	C(8a)–N(9)	1.389(7)
C(8a)–C(4b)	1.410(7)	N(9)–C(9a)	1.386(7)
C(9a)–S(1)	1.740(6)	C(9a)–C(4a)	1.362(6)
S(1)–C(2)	1.815(7)	C(2)–C(3)	1.523(7)
C(3)–C(4)	1.540(7)	C(4)–C(4a)	1.500(6)
C(4)–C(10)	1.528(7)	C(4a)–C(4b)	1.445(6)
C(10)–N(11)	1.488(7)	N(11)–C(12)	1.498(8)
C(6)–C(5)–C(4b)	117.2(4)	C(5)–C(6)–C(7)	121.5(5)
C(6)–C(7)–C(8)	121.4(5)	C(7)–C(8)–C(8a)	117.1(5)
C(8)–C(8a)–N(9)	129.5(5)	C(8)–C(8a)–C(4b)	122.5(5)
N(9)–C(8a)–C(4b)	107.9(4)	C(8a)–N(9)–C(9a)	108.0(4)
N(9)–C(9a)–S(1)	120.3(4)	N(9)–C(9a)–C(4a)	110.5(4)
S(1)–C(9a)–C(4a)	129.2(4)	C(9a)–S(1)–C(2)	97.2(3)
S(1)–C(2)–C(3)	114.0(4)	C(2)–C(3)–C(4)	113.4(4)
C(3)–C(4)–C(4a)	110.5(4)	C(3)–C(4)–C(10)	114.2(4)
C(4a)–C(4)–C(10)	108.3(4)	C(9a)–C(4a)–C(4)	125.4(4)
C(9a)–C(4a)–C(4b)	106.6(4)	C(4)–C(4a)–C(4b)	127.9(4)
C(5)–C(4b)–C(8a)	120.2(4)	C(5)–C(4b)–C(4a)	133.0(4)
C(8a)–C(4b)–C(4a)	106.9(4)	C(4)–C(10)–N(11)	113.0(4)
C(10)–N(11)–C(12)	112.9(4)		

Each diastereoisomeric amide afforded a single peak at 8.4 min for (*SR*)-(**25**) and 9.2 min for (*RR*)-(**25**). Excellent optical purities were thus demonstrated.

Determination of Absolute Configuration.—Starting from (*S*)-malic acid, single inversion of the configuration, but not double inversion, occurred once between (*S*)-(**9**) and (+)-(**11**),[2] which led to optically pure (+)-(**1**) and (+)-(**2**). Furthermore, the stereoselectivity of the cyclization was as much as 97% by comparison with the enantiomeric excess of (**8**) and (**11**). Hence the mechanism of the crucial cyclization step was fully established,[2] (+)-(**11**), and thus (+)-(**1**) and (+)-(**2**) then having the (*S*)-configuration. Further confirmation of the absolute configuration was obtained from a single crystal X-ray crystallographic analysis of the *p*-bromobenzylamine derivative (**19**) prepared from (+)-(**1**) (Figure 2).

X-Ray Crystallography.—The crystal structure of (**2**) was examined as the HCl salt, (**2**) · HCl. The atomic fractional co-ordinates, bond lengths, and bond angles are listed in Tables 1 and 2.*

The X-ray crystallographic study showed that in the thiopyrano ring of (**2**)·HCl, the atoms (C4), C(4a), C(9a), and S(1) are almost coplanar; the RMS deviations from the mean plane through the four atoms is 0.016 Å. C(2) and C(3) Deviate from the mean plane by 0.305 and −0.447 Å, respectively and the ring, therefore, adopts a half-chair conformation. The torsion angle of C(10)–C(4)–C(4a)–C(5) is 72.5°, and thus the orientation of the C(10) group is pseudoaxial. The methylaminomethyl side chain at C(4) is almost in the fully extended conformation with respect to N(11)–C(10)–C(4)–C(4a) and C(12)–N(11)–C(10)–C(4), respectively (Figure 3).

The structure of (**19**) was solved by the heavy-atom method. C(4), C(4a), C(9a), and S(1) Are also near coplanar; the RMS deviation from the mean plane through these atoms is 0.021 Å. The C(2) atom is disordered and can be resolved in two positions, one being at the upper side and the other at the lower side of the mean plane of C(4), C(4a), C(9a), and S(1) by 0.439 and −0.700 Å, respectively, which are presented by C(2) and C(2′) in Figure 2. The occupancy factors were estimated to be 0.5 based on the peak heights at each position in the difference electron density map. The temperature factors of S(1) and C(3) exhibited large anisotropy, but their disordered positions could not be distinguished on the map. Deviation of C(3) from the mean plane was *ca.* −0.12 Å. The torsion angle of C(10)–C(4)–C(4a)–C(5) is 64.1°, which is smaller than that of (**2**)·HCl by *ca.* 8°.

The N(11) atom and indole ring are *trans*, and C(12) and C(4) have a gauche relationship with respect to the relevant axes. The aromatic ring of the benzyl group lies above the 5(H) of the indole ring to form a perpendicular edge-to-face arrangement: the angle of two mean planes of six-membered aromatic rings and distance between these two centroids are 82.4° and 5.14 Å, respectively.

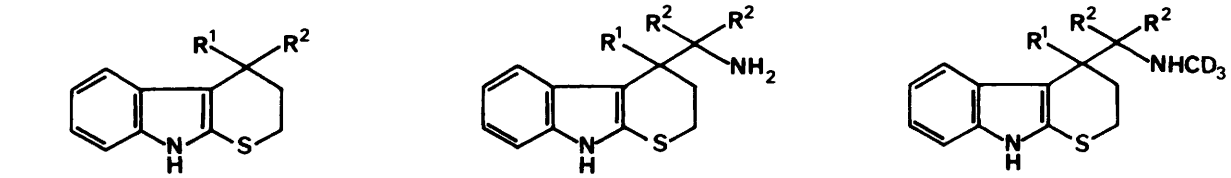
Though the bond lengths and angles relevant to C(2) and C(2′) are less accurate, hydrogen atoms except for those attached to C(2), C(2′), and C(3), were identified. The atomic fractional co-ordinates, and bond lengths and bond angles of (**19**) are listed in Tables 3 and 4.* Selected torsion angles of both (**2**)·HCl and (**19**) are tabulated in Table 5.

NMR Spectroscopy.—^{1}H NMR spectra† of (**2**) were measured in $CDCl_3$ at 200 MHz, and in [$^2H_5$]pyridine at 90 MHz, respectively. The spectra were difficult to interpret because of several overlapping resonances. In order to analyse the aliphatic hydrogens (2-H, 3-H, 4-H and 10-H), deuterium-labelled compounds, [10-$^2H_1$]-(**2**), [4, 10-$^2H_2$]-(**2**), and [4-$^2H_1$]-(**2**) were prepared as depicted in Scheme 4. These spectra allowed unambiguous assignments of all aliphatic proton resonances around the thiopyrano ring entity (C ring)‡ at 200 MHz; however, the 10(H) coupling constants remained undetermined at 90 MHz owing to their higher order couplings. Protons attached to C(10) and C(4) could be analysed as an ABX system where A and B were diastereotopic methylene protons at C(10) (δ 2.82 and 3.02, ^2J = 11.8, ^3J = 8.6, and 4.9 Hz), and X is 4-H (δ 3.32). The initial parameters were refined by the program LAOCN3.[8] The results are shown in Table 6, with

* Bond lengths, bond angles, and thermal parameters for (**2**)·HCl and (**19**) have been deposited at the Cambridge Crystallographic Data Centre. See Instructions for Authors, *J. Chem. Soc., Perkins Trans. 1*, 1989, Issue 1.

† Details of assignments of ^{1}H and ^{13}C NMR signals are described in our preceding paper (ref. 2).

‡ For the convenience of abbreviation, the terms A, B, and C ring are here used to denote the six- and five-membered rings of the indole and the thiopyrano ring, respectively, in these tricyclic indole ring systems.



Scheme 4.

Table 3. Atomic co-ordinates ($\times 10^4$) with esds in parentheses for (**19**)[a]

	x	*y*	*z*
S(1)	−1 216(1)	5 446(1)	885(3)
C(2)	−824(13)	4 248(10)	177(19)
C(2′)	−1 002(15)	4 285(11)	1 367(25)
C(3)	−94(7)	3 828(6)	914(14)
C(4)	966(5)	4 273(4)	1 250(8)
C(4a)	904(5)	5 321(4)	1 216(6)
C(4b)	1 737(5)	5 962(4)	1 355(7)
C(5)	2 795(5)	5 851(5)	1 470(8)
C(6)	3 375(7)	6 673(6)	1 600(10)
C(7)	2 958(7)	7 521(6)	1 557(9)
C(8)	1 884(7)	7 658(5)	1 397(8)
C(8a)	1 297(5)	6 850(4)	1 331(7)
N(9)	260(4)	6 756(4)	1 190(6)
C(9a)	42(5)	5 825(4)	1 140(7)
C(10)	1 373(6)	3 967(4)	2 710(8)
N(11)	1 525(5)	2 953(4)	2 868(7)
C(12)	2 191(7)	2 523(5)	1 787(9)
C(13)	3 175(6)	3 048(5)	1 497(7)
C(14)	3 833(7)	3 305(6)	2 596(8)
C(15)	4 700(6)	3 815(7)	2 366(9)
C(16)	4 939(6)	4 064(5)	1 017(8)
C(17)	4 350(6)	3 804(5)	−119(8)
C(18)	3 464(6)	3 284(5)	143(8)
Br	6 123.5(8)	4 801.9(9)	669.3(15)

[a] Atom numbers are shown in Figure 1. C(12) to C(18) and Br are assigned to the atoms in *p*-Br-benzyl group.

Table 4. Bond lengths (Å) and angles (°) with esds in parentheses for (**19**).

C(5)–C(6)	1.412(12)	C(5)–C(4b)	1.394(10)
C(6)–C(7)	1.339(13)	C(7)–C(8)	1.424(13)
C(8)–C(8a)	1.396(11)	C(8a)–N(9)	1.367(9)
C(8a)–C(4b)	1.404(9)	N(9)–C(9a)	1.373(9)
C(9a)–S(1)	1.747(7)	C(9a)–C(4a)	1.341(9)
S(1)–C(2)	1.921(18)	S(1)–C(2′)	1.757(24)
C(2)–C(3)	1.325(22)	C(2′)–C(3)	1.421(27)
C(3)–C(4)	1.557(15)	C(4)–C(4a)	1.514(10)
C(4)–C(10)	1.538(11)	C(4a)–C(4b)	1.433(9)
C(10)–N(11)	1.483(10)	N(11)–C(12)	1.475(11)
C(12)–C(13)	1.516(12)	C(13)–C(14)	1.394(12)
C(13)–C(18)	1.372(11)	C(14)–C(15)	1.367(14)
C(15)–C(16)	1.356(13)	C(16)–C(17)	1.369(11)
C(16)–Br	1.905(8)	C(17)–C(18)	1.400(11)
C(6)–C(5)–C(4b)	116.2(7)	C(5)–C(6)–C(7)	123.1(8)
C(6)–C(7)–C(8)	122.0(9)	C(7)–C(8)–C(8a)	115.4(8)
C(8)–C(8a)–N(9)	129.1(7)	C(8)–C(8a)–C(4b)	122.4(7)
N(9)–C(8a)–C(4b)	108.4(6)	C(8a)–N(9)–C(9a)	107.8(6)
N(9)–C(9a)–S(1)	120.4(5)	N(9)–C(9a)–C(4a)	110.8(6)
S(1)–C(9a)–C(4a)	128.8(5)	C(9a)–S(1)–C(2)	94.5(6)
C(9a)–S(1)–C(2′)	96.5(8)	S(1)–C(2)–C(3)	114.9(12)
S(1)–C(2′)–C(3)	119.8(16)	C(2)–C(3)–C(4)	123.8(12)
C(2′)–C(3)–C(4)	119.2(13)	C(3)–C(4)–C(4a)	111.1(7)
C(3)–C(4)–C(10)	111.7(7)	C(4a)–C(4)–C(10)	108.9(6)
C(9a)–C(4a)–C(4)	125.9(6)	C(9a)–C(4a)–C(4b)	106.9(6)
C(4)–C(4a)–C(4b)	127.0(6)	C(5)–C(4b)–C(8a)	120.7(6)
C(5)–C(4b)–C(4a)	133.2(6)	C(8a)–C(4b)–C(4a)	106.1(6)
C(4)–C(10)–N(11)	114.8(6)	C(10)–N(11)–C(12)	115.1(6)
N(11)–C(12)–C(13)	114.4(7)	C(12)–C(13)–C(14)	121.4(7)
C(12)–C(13)–C(18)	121.6(7)	C(14)–C(13)–C(18)	117.0(7)
C(13)–C(14)–C(15)	122.4(9)	C(14)–C(15)–C(16)	118.8(9)
C(15)–C(16)–C(17)	122.0(8)	C(15)–C(16)–Br	119.7(7)
C(17)–C(16)–Br	118.3(6)	C(16)–C(17)–C(18)	118.2(7)
C(13)–C(18)–C(17)	121.6(7)		

the arithmetic means for the coupling constants of 2-H to 4-H of six 4-substituted tetrahydrothiopyrano[2,3-*b*]indoles[2] in $CDCl_3$ included for comparison.

^{13}C NMR data* were obtained in $CDCl_3$ and are shown in Table 7. The data for (**27**)[2] are also included for comparison.

Pharmacology.—The analgesic activities of both enantiomers of (**2**) were compared by oral administration by the mouse acetic acid writhing (AAW),[9] phenylbenzoquinone-induced writhing (PQW),[10] and modified Haffner method.[11] The results are summarized in Table 8.

Compound (*R*)-(**2**) had an ED_{50} of 5.5 mg/kg in AAW, being 4-fold more potent than (*S*)-(**2**). In the PQW assay, the (*R*)-enantiomer with the ED_{50} of 8.8 mg/kg was 6-fold more potent than the (*S*)-isomer. Compound (*R*)-(**2**) was 19 times more potent than (*S*)-(**2**) in the modified Haffner method with an ED_{50} of 4.8 mg/kg.

Discussion

Pharmacophore models include the notion of symmetrical fitting between a macromolecular receptor and a small drug molecule; the receptor recognizes the pharmacophoric pattern of a substrate just as the substrate recognizes a complementary receptor pattern; there is also the possibility of flexibility in each partner.[12] This concept implies the roles of absolute configuration and conformation of a drug which may cause or enhance the biological activity under physiological conditions.

We have synthesized both enantiomers of compound (**2**) starting from (*S*)-malic acid and oxindole by way of an indole Grignard reaction which we had improved to use as an intramolecular reaction.[2] The crucial cyclization reactions of both (*S*)- and (*R*)-enantiomers of the sulphides (**9**) had indeed proceeded regio- and stereo-specifically to give the desired tricyclic intermediates which led to enantiomerically pure (*S*)- and (*R*)-(**2**) after several functionalizations. Based on the reaction mechanism,[2] the absolute configurations were deter-

* Details of assignments of ^1H and ^{13}C NMR signals are described in our preceding paper (ref. 2).

Table 5. Selected torsion angles of (2)·HCl and (19).

Torsion angle	(2)·HCl	(19)
C(10)–C(4)–C(4a)–C(4b)	71.60	62.13
N(11)–C(10)–C(4)–C(4a)	−163.57	−176.96
C(12)–N(11)–C(10)–C(4)	175.08	55.17
C(13)–C(12)–N(11)–C(10)	—	46.0
C(9a)–S(1)–C(2)–C(3)	−41.16	−40.71
C(9a)–S(1)–C(2′)–C(3)	—	45.14[a]
S(1)–C(2)–C(3)–C(4)	66.26	54.29
S(1)–C(2′)–C(3)–C(4)	—	−54.38[a]
C(2)–C(3)–C(4)–C(4a)	−52.17	−33.76
C(2′)–C(3)–C(4)–C(4a)	—	24.39[a]
C(3)–C(4)–C(4a)–C(4b)	−162.61	−174.44
C(4)–C(4a)–C(9a)–S(1)	−5.17	−6.95
C(4a)–C(9a)–S(1)–C(2)	13.16	18.57

[a] See text and Figure 2.

In compound (2), each of the coupling constants in the CH_2CH_2CH fragment in both $CDCl_3$ and [$^2H_5$]pyridine solvents is almost identical with that of other 4-substituted thiopyrano[2,3-*b*]indoles (Table 6).[2] Furthermore, the resonance of C(2) was shielded by −3.7 ppm in ^{13}C NMR than that of the unsubstituted compound (27), reflecting the steric γ-gauche effect of the pseudoaxial C(10) group.[2] Based on an NMR spectroscopic study, we have already described that the common and predominant conformation of the C rings of 4-substituted thiopyrano[2,3-*b*]indoles is the half-chair conformation and that C(10) is located in a pseudoaxial position.[2] The present NMR data clearly indicate that the C ring geometry of (2) in solution is identical with that of other 4-substituted analogues. Furthermore, the crystal structure of (2)·HCl almost perfectly corresponds to the shape predicted by NMR spectroscopy.

In the crystal structure of (19), however, two kinds of C(2) envelope conformers were found in almost equal amount; one is analogous to the structure of (2) shown by NMR and X-ray crystallography, and the other different. This suggests that two energetically indistinguishable conformers could exist in (19) and, therefore, (2).

Burley and co-workers reported the significant contributions of intra- and inter-molecular edge-to-face aromatic-aromatic interactions to stabilize the structures of proteins and small biphenylic peptides.[13a–c,14] We found that the perpendicular disposition of the six-membered rings of the indole and benzyl groups in the crystal structures of (19) is strikingly similar to the corresponding entities of the crystal structures of *N*-phenylacetyl-L-Phe,[14a] L-Lys-L-Phe-L-Phe,[14b] and the benzoyl ester of L-Phe,[14c] which Burley *et al.* had demonstrated as examples of such aromatic-aromatic interactions.[13a] Figure 5 illustrates the comparison of the crystal structures of (19) and these peptides[14]

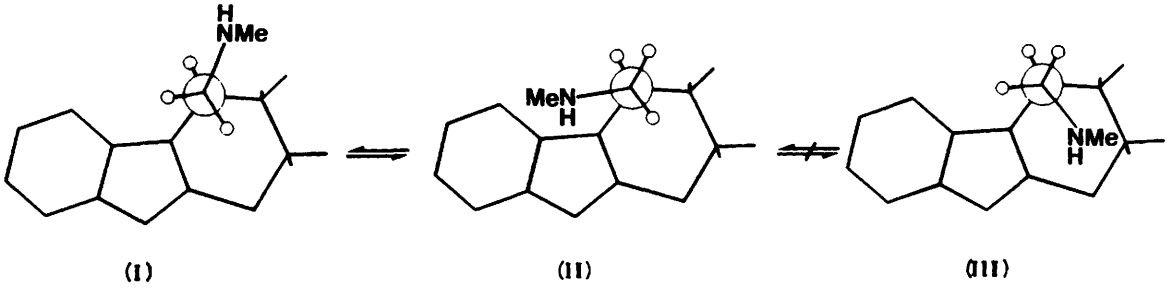
Table 6. ^1H NMR data of (2) in $CDCl_3$[a] and [$^2H_5$]pyridine[b].

	Chemical shifts[c]							Coupling constants (Hz)[c,d]										
Solvent	2e	2a	3e	3a	4	10	10′	2e–2a	2e–3e	2e–3a	2a–3a	2a–3e	3e–3a	3e–4	3a–4	10–10′	4–10	4–10′
$CDCl_3$	2.97	3.31	2.40	2.10	3.32	3.02	2.82	12.8	6.1	2.8	11.2	2.4	14.1	4.3	5.7	11.8	8.6	4.9
[$^2H_5$]Pyridine	2.92	3.38	2.54	2.06	3.42		*e*	12.8	5.9	2.7	11.5	2.3	14.0	3.6	5.5		*e*	
Mean[f]								12.8	6.6	2.8	10.9	2.3	14.1	4.5	5.5			

[a] Measured at 200 MHz. [b] Measured at 90 MHz. [c] All the parameters were refined by LAOCN3. RMS deviations between experimental and calculated spectra were within 0.2 Hz. [d] Absolute values. [e] Not determined. [f] Arithmetic means of six 4-substituted thiopyrano[2,3-*b*]indoles. See Ref. 2.

Table 7. ^{13}C NMR data of (2) in $CDCl_3$.

	C(2)	C(3)	C(4)	C(4a)	C(4b)	C(5)	C(6)	C(7)	C(8)	C(8a)	C(9a)	C(10)	NMe
(2)	24.5	27.0	31.4	108.3	127.3	116.7	119.4	120.6	110.1	136.1	128.2	56.4	36.6
(27)	28.2	23.6	20.5	107.2	125.9	116.3	119.4	120.8	109.9	135.7	128.5		



(I) (II) (III)

Figure 4. Newman projections of three perfectly staggered rotamers of side-chain amino group along the C(4)-C(10) bond. Aromatic hydrogens were removed for clarity.

Table 8. Analgesic activities of (*S*)- and (*R*)-(2).

Method[a,b]	(*S*)-(2)	(*R*)-(2)
AcOH writhing:	20.8 (18.2–23.9)	5.5 (4.8–6.2)
PQ writhing:	55.6 (41.2–74.2)	8.8 (6.0–11.9)
Modified Haffner:	92.2 (80.9–106.7)	4.8 (3.9–5.6)

[a] AcOH = acetic acid, PQ = phenylbenzoquinone. [b] ED_{50} (mg/kg) *p.o.* (95% confidence limits).

mined, which was unambiguously supported by X-ray crystallography. Compound (*R*)-(2) was shown to exert 4 to 19 times more potent analgesic activity than the (*S*)-isomer in the preliminary assays.

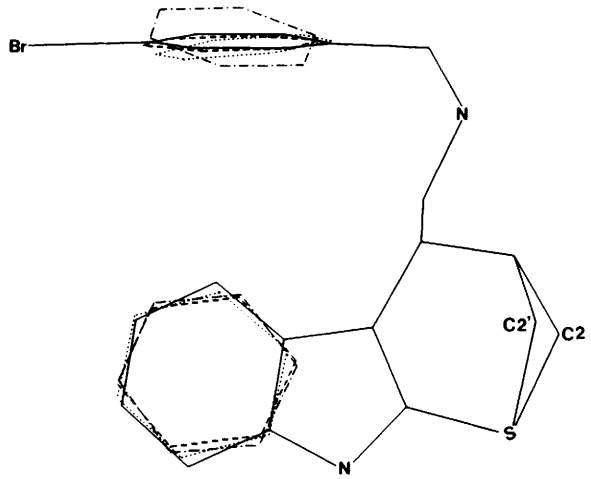


Figure 5. Comparison of (**19**) (bold line) and *N*-phenylacetyl-L-Phe- (– – – –), L-Lys-L-Phe-L-Phe (— · — · —), and benzoyl ester of L-Phe (· · · ·). Each peptide molecule was superimposed to (**19**) by fitting at 12 points of two phenyl rings. Only phenyl rings are shown for clarity. The co-ordinates of these peptides were obtained from ref. 14*a–c*.

Table 9. ^{13}C Spin-lattice relaxation time of (**2**).

Carbon	T_1	NT_1	NOE	T^{DD}
2	0.49	0.98	2.02	0.49
3	0.50	1.00	1.90	0.52
4	0.94	0.94	1.85	1.01
4a	18.0	18.0	1.76	20.3
4b	20.0	20.0	1.55	25.7
5	0.72	0.72	1.99	0.72
6	0.88	0.88	2.00	0.88
7	0.79	0.79	2.08	0.79
8	0.78	0.78	1.81	0.86
8a	17.7	17.7	1.84	19.2
9a	21.4	21.4	1.77	24.1
10	0.60	1.2	1.94	0.60
N-Me	1.70	5.1	1.89	1.78

by means of least-square fitting of the aromatic entities. The distance (5.1 Å) and dihedral angle (82.4°) between two six-membered aromatic rings of (**19**) certainly suggest that the side-chain entity of (**19**) is stabilized by the typical intramolecular edge-to-face aromatic-aromatic interaction that Burley *et al.* proposed (Figure 2).[13]

Whereas, the torsion angle of C(10)–C(4)–C(4a)–C(5) in (**19**) indicates that the orientation of the C(10) group is still pseudoaxial. The distances between C(5) and C(10) are 3.64 Å for (**2**)·HCl and 3.50 Å for (**19**), van der Waals contacts thus being maintained. If, instead, the C(10) group is pseudoequatorial, the C(10) group would suffer from poor van der Waals contact with 5(H), as is readily shown by a CPK model. This must force the orientation of C(10) pseudoaxially, and consequently anchor the C ring geometry.

We consider that the pseudo-cyclic[13b] partial structure through the C(H)-5 portion of the indole ring to the benzyl group must result in modification of the C ring properties of (**19**) to yield a further unusual conformer; the preferred C ring geometry of (**2**) must be like that presented by NMR spectroscopy and the crystal structure of (**2**)·HCl.

The existence of a predominant conformer was qualitatively supported by the ^{13}C spin-lattice relaxation time (T_1) and nuclear Overhauser effect (NOE)[15] of (**2**) (Table 9). The observed full NOE enhancements indicate that the relaxation of these protons would be dominated by a dipole-dipole mechanism. The T_1's of the whole protonated carbons on the rings were < 1.0 s, and the NT_1 values of C(2) (0.98), C(3) (1.0), C(4) (0.94), and the protonated aromatic carbons (0.72–0.88) are close to each other. For a rigid molecule tumbling isotropically in solution, NT_1 is expected to be equal for each protonated carbon. This is the case suggesting the restricted internal motion of the C ring.

The conformation of the side chain of (**2**) and, therefore, the overall three-dimensional structure, can most probably be described in terms of the fractional populations of the three perfectly staggered rotamers, (**I**), (**II**), and (**III**), (see Figure 4) when the predominant orientation of the C(10) group is pseudoaxial.

In the crystal structures, both (**2**)·HCl and (**19**) were shown to adopt conformer (**I**). Conformations in solution are often estimated from the coupling constants of relevant hydrogens as the averaged contribution of the conformers weighted according to the fractional populations. In this case, the contribution of (**III**) seems to be less significant since its side chain lies over the heterocyclic rings, and it is, therefore, obviously energetically unfavourable. Therefore, following Snyder's equation (1),[16] we estimated the approximate fractional populations P(**I**) and P(**II**) to be 70:30, or inversely 30:70, though it was impossible to conclude definitively which was the most preferred one since it was not possible to differentiate between 10-H and 10-H′.

$$P(\mathbf{I})/P(\mathbf{II}) = (\gamma\text{-}0.18)/(1\text{-}0.18\gamma) \quad (1)$$
where $\gamma = J/J' = 8.6/4.9$ or alternatively 4.9/8.6.

Burley and co-workers pointed out that the contribution of an interaction weaker than that of a hydrogen-bond might play a significant role in biological activity.[13] It is noteworthy, that the C(H)-5 region is demonstrated to act as a counterpart to intramolecular aromatic-aromatic interaction, a finding which could be an important pointer both in drug design and for predicting possible interactions between the active sites of a receptor and a drug. That is, in compound (**2**) this region could interact with an aromatic residue, *e.g.* Phe or Tyr, of an active site of analgesic activity intermolecularly instead of by intramolecular association; the aromatic group found in the crystal structure of (**19**) demonstrates this. If the aromatic counterpart in a protein were Tyr, the hydroxy group of Tyr and N(11) of (**2**) have the possibility of forming a hydrogen bond simultaneously. Figure 6 depicts the putative complex of (**2**) and a Tyr residue as an example (Tyr is represented by a simple phenol molecule). The Figure shows that both molecules could associate by means of hydrogen bond between N(11) of (**2**) and the hydroxy group of a Tyr residue and edge-to-face aromatic-aromatic interaction between two aromatic rings to form a pseudo-cyclic complex, which is quite similar to that of (**19**) (Figure 2). These dual interactions could serve as a scaffold to enhance analgesic activity by not only tightening the association but also directing the orientation of a drug to ensure more suitable interactions between its other pharmacophores and the receptor, as, for example, the indole nitrogen of (**2**) as hydrogen-bond site.[17] It should be pointed out here that the introduction of substituents into the 4- or 5-position of indoles (indole numbering) often enhances the pharmacological activity and, in fact, many biologically important indole derivatives carry the substituents at these positions. However, the structure-activity relationships of (**2**)[1] indicated that introduction of substituents into the corresponding 5- or 6-position of (**2**) significantly reduced the analgesic activity compared with that of (**2**) itself. These findings partly support our present hypothesis because introduction of substituents into these positions obviously interferes with the aromatic-aromatic interaction.

In order to form a rigid pseudo-cyclic complex, conformer (**I**)

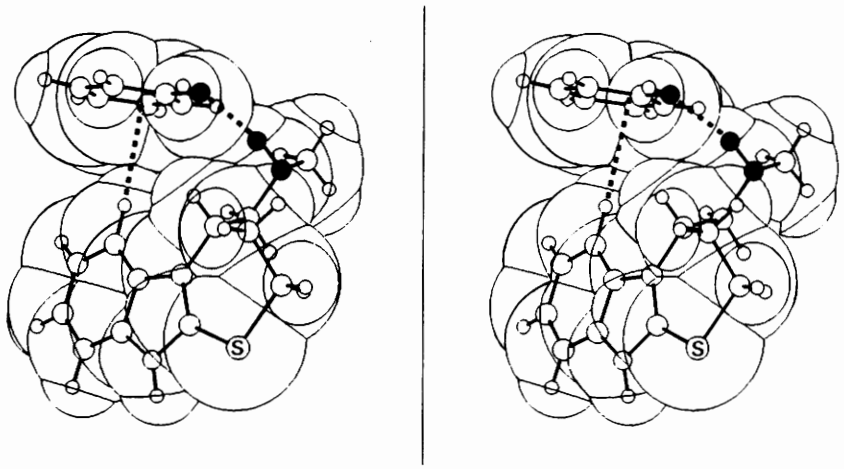


Figure 6. A model for the proposed interaction between (*R*)-(**2**) and Tyr being presented by the phenol molecule. Two rings are bridged by the N–H–O hydrogen bond and edge-to-face aromatic–aromatic interaction (dotted lines). ● denotes N, H, and O atoms. These structures were obtained from the crystal structure of (*S*)-(**2**) and phenol derivative. The following distances and angles were used in constructing this complex: N–H–O, 3.0 Å; two centroids of six-membered ring, 5.3 Å; angle between the rings, 83.8°.

must be the preferred structure and we have demonstrated that (**2**) has sufficient chance to adopt this structure.

The C(H)-5 region has been shown to play a role in regulating the conformation of tetrahydrothiopyrano[2,3-*b*]indoles including (**2**). Interestingly, the present study further suggests that this region could play an additional role in interaction with the active site. Since both configurational and conformational properties of (**2**) were shown, the results would be able to provide one useful model as a 'probe for the active site' of an analgesic.

Experimental

M.p.s and b.p.s are uncorrected. IR spectra were recorded on a Hitachi Model 260–10 instrument. ^{1}H NMR spectra were recorded on a Varian XL-200 (200 MHz) or EM-390 (90 MHz) machines with tetramethylsilane as the internal standard. Optical rotations were measured in a 10-cm cell on a Perkin-Elmer 141 polarimeter. Analytical HPLC was conducted on a Waters 6000A instrument with the UV detector at 254 nm. Column chromatography was performed on Merck silica gel 60 (230–400 mesh). All the reactions were carried out under an atmosphere of nitrogen. Commercially available ether solution of EtMgBr (*ca.* 3M) was used as received. Unless otherwise stated, the organic extracts were washed with saturated brine, dried ($MgSO_4$), and concentrated under reduced pressure. Computations were performed on VAX-11/780 and PS300 (E & S) instruments using MOLOG[18a] and MOGLI[18b] software.

(S)-(+)-3,4-O-*Isopropylidenedioxybutan*-1-*ol* (**3**).—The title compound (**3**) was prepared from (*S*)-malic acid (170 g, 1.27 mol) according to the literature procedure[3] (50% overall yield): b.p. 112 °C (24 mmHg), $[\alpha]_D^{23}$ + 0.6° (*c* 3.60, EtOH). The $[\alpha]_D$ value of (**3**) was lower than that of (*R*)-(**3**) reported (lit.,[3] $[\alpha]_D$ −3.7° (*c* 3.7, EtOH)). Attempts to increase $[\alpha]_D$ were unsuccessful. However, (**3**) has a satisfactory enantiomeric purity as reported below.

(S)-(−)-1-*Bromo*-3,4-O-*isopropylidenedioxybutane* (**4**).—A mixture of (**3**) (84 g, 0.575 mol) and tosyl chloride (120 g, 0.628 mol) was stirred at 3 °C in pyridine (800 ml) for 16 h. The solvent was removed under reduced pressure, and the residue was extracted. Work-up afforded the tosylate (138 g), which was treated with anhydrous LiBr (100 g, 1.15 mol) at 85 °C in DMF (900 ml). After 2.5 h, the mixture was poured into ice–water (1 l), and the layers were extracted with ether. The crude material was distilled to give (**4**) (65 g, 54%): b.p. 96–100 °C (26 mmHg); $[\alpha]_D^{23}$ − 26.4° (*c* 8.40, EtOH).

(S)-(−)-2-(3,4-*Dihydroxybutylthio*)*indole* (S)-(**7**).—A mixture of (**5**) (31.5 g, 0.21 mol), K_2CO_3 (30.0 g, 0.22 mol), and the bromide (**4**) (45.1 g, 0.22 mol) was stirred at 23 °C in acetone (310 ml) for 4 h. After this the solvent was removed under reduced pressure and the residue worked up to afford (**6**) (59 g) as a dark oil. This was dissolved in MeOH (650 ml), and 3M HCl (130 ml) was added. The mixture was stirred at 23 °C for 15 min after which the solvent was removed under reduced pressure. The residue was extracted with EtOAc and the extract worked up to afford a yellow oil (48.0 g), which was roughly purified by silica gel (220 g) chromatography. The less polar products were removed with EtOAc–benzene (2:98). Continued elution with EtOAc afforded (**7**) [43 g, 84% from (**5**)] as a colourless viscous oil: $[\alpha]_D^{23}$ − 22.3° (*c* 2.39, $CHCl_3$); ν_{max}($CHCl_3$) 3 580, 3 450 (NH), and 3 400–3 100br cm^{-1}.

(S)-(−)-2-(4-*t*-*Butyldimethylsilyloxy*-3-*hydroxybutylthio*)-*indole* (S)-(**8**).—t-Butyldimethylchlorosilane (25.5 g, 0.17 mol) was added to a solution of (**7**) (40 g, 0.17 mol), DMAP (2.0 g, 0.016 mol), and Et_3N (50 ml, 0.36 mol) in CH_2Cl_2 (800 ml) at −13 °C. The mixture was stirred at the same temperature for 3 h after which it was poured into ice. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. Work-up and purification of the extract by silica gel chromatography (600 g, EtOAc–hexane, 1:4) afforded (*S*)-(**8**) (48.1 g, 81%): viscous oil; $[\alpha]_D^{23}$ − 16.8° (*c* 3.01, $CHCl_3$); ν_{max}($CHCl_3$) 3 450 cm^{-1} (NH); δ_H(90 MHz; $CDCl_3$) 0.03 (6 H, s, Me_2Si), 0.81 (9 H, s, Bu^t), 1.62 (2 H, m, CH_2), 2.61 (1 H, d, *J* 4.5 Hz, OH), 2.90 (2 H, m, SCH_2), 3.25–3.77 (2 H, m, OCH_2), 3.75 (1 H, m, CH), 6.52 (1 H, d, *J* 2 Hz, NC=CH), 6.9–7.5 (4 H, m, ArH), and 8.35 (1 H, br, NH).

(R)-(+)-2-(4-t-*Butyldimethylsilyloxy*-3-*hydroxybutylthio*)-*indole* (R)-(**8**).—To a solution of (*S*)-(**8**) (24.2 g, 69 mmol), triphenylphosphine (25.4 g, 97 mmol), and *o*-acetylsalicylic acid (17.4 g, 97 mmol) in THF (480 ml) at −13 °C was added dropwise diethyl azodicarboxylate (15.2 ml, 97 mmol). The mixture was stirred at −13 °C for 1 h and then at 3 °C for 30

min. The solvent was removed below 10 °C under reduced pressure and the residue was extracted with ether. The ether layer was washed with aqueous $NaHCO_3$ and brine and then dried and concentrated. The residue was triturated with a small amount of benzene, and the precipitate was removed. The filter cake was washed with benzene, and the combined filtrates were concentrated. Purification of the residue on silica gel (200 g; benzene) yielded the salicylate (**22**) (30.4 g, 86%) as a colourless oil; $[\alpha]_D^{23}$ + 8.35° (*c* 3.16, $CHCl_3$); δ_H(90 MHz; $CDCl_3$) 0.03 (6 H, s, Me_2Si), 0.85 (9 H, s, Bu^tSi), 1.8–2.3 (2 H, m, CH_2), 2.28 (3 H, s, OAc), 2.83 (2 H, t, *J* 7 Hz, SCH_2), 3.72 (2 H, d, *J* 5 Hz, CH_2OSi), 5.30 (1 H, m, CH), 6.62 (1 H, d, *J* 2 Hz, NC=CH), and 6.9–8.1 (8 H, m, ArH); ν_{max}($CHCl_3$) 3 590, 3 445 (NH), 3 340, 1 750, and 1 710 cm^{-1}.

A solution of (**22**) (30.4 g, 59 mmol) in ether (300 ml) was added to a suspension of $LiAlH_4$ (6.30 g, 0.166 mol) in ether (300 ml) at −65 °C over a 30 min period. Stirring was continued for an additional 30 min after which water (64 ml) was added carefully. The resulting colourless slurry was allowed to warm to 0 °C when the precipitate was filtered off and washed well with ether. The ether layers were dried and concentrated and the residue was purified over silica gel (190 g) with EtOAc–benzene (2:98) as eluant to afford (*R*)-(**8**) [19.4 g, 80% from (*S*)-(**8**)] as a colourless oil; $[\alpha]_D^{23}$ + 17.2 (*c* 3.10, $CHCl_3$). The spectral properties of (*R*)-(**8**) were identical in all respects with those of (*S*)-(**8**).

Experimental details for the preparation of (R)-*enantiomers in the following sections were omitted since they were well represented by those described for the corresponding* (S)-*isomers. Prefixes to specify absolute configuration were also omitted. Only the physical constants were presented for the* (R)-*isomers.*

4-*t-Butyldimethylsiloxymethyl*-2,3,4,9-*tetrahydrothiopyrano*-[2,3-b]*indole* (**10**).—*Method A.* Methanesulphonic anhydride (MSA) (8.9 g, 51 mmol) was added to a solution of compound (**8**) (15 g, 43 mmol) and Et_3N (13 ml, 93 mmol) in CH_2Cl_2 (150 ml) at −13 °C. The mixture was stirred for 15 min and then poured into water. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed, dried, and concentrated to yield the mesylate (**9**) (11.0 g) as an oil, which was used without purification. In a smaller scale reaction, spectroscopically pure (**9**) was obtained by silica gel (10 g) chromatography (EtOAc–benzene, 5:95): $[\alpha]_D^{23}$ + 0.6 (*c* 1.02, $CHCl_3$); δ_H(90 MHz; $CDCl_3$) 0.04 (6 H, s, Me_2), 0.85 (9 H, s, Bu^t), 1.93 (2 H, m, CH_2), 2.91 (2 H, t, *J* 7.5 Hz, SCH_2), 3.03 (3 H, s, Ms), 3.71 (2 H, m, CH_2OSi), 4.91 (1 H, m, CH), 6.63 (1 H, d, *J* 2 Hz, NC=CH), 7.0–7.6 (4 H, m, ArH), and 8.45 (1 H, br, NH).

To a solution of unpurified (**9**) in benzene (150 ml) at 10 °C was added dropwise an ether solution of EtMgBr (3.10 M; 30 ml, 93 mmol). The resulting yellow suspension was stirred for 10 min and then an aqueous solution of NH_4Cl was added carefully. The layers were separated and the aqueous layer was extracted with ether. The organic layers were washed, dried, and concentrated to give (**10**) (9.0 g) as a colourless oil; *m/z* 333 (M^+, 10%), 276(5), and 188 (100). This material was used without purification in the next reaction.

Method B: one-pot procedure. MSA (146 mg, 0.84 mmol) was added to a stirred solution of (**8**) (280 mg, 0.8 mmol) and Et_3N (0.13 ml, 0.93 mmol) in CH_2Cl_2 (6 ml) at −13 °C. After 10 min, an ether solution of EtMgBr (2.7 M; 1 ml, 2.7 mmol) was added dropwise. The mixture was stirred for 10 min. An aqueous solution of NH_4Cl was added, and the layers were extracted with ether. The organic layers were washed, dried, and concentrated to give (**10**) (280 mg, 95%).

4-*Hydroxymethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**11**).—A mixture of (**10**) (257 mg, 0.77 mmol) and *p*-TsOH (monohydrate, 20 mg) was stirred in methanol (5 ml) at 23 °C for 30 min. Excess of $NaHCO_3$ was added in one portion, and the solvent was removed under reduced pressure. The residue was extracted with ether and the extracts were washed, dried, and concentrated. Purification of the residue by silica gel (20 g; EtOAc–hexane, 1:4) gave (**11**) (144 mg, 85%) as a colourless oil; $[\alpha]_D^{23}$ + 55.0 (*c.* 4.59, $CHCl_3$). IR and NMR spectra were identical with those of racemic (**11**).[2]

9-*Acetyl*-4-*hydroxymethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**14**).—Unpurified (**10**) (18.2 g, 54.6 mmol) was treated with acetic anhydride (25 ml, 265 mmol) and DMAP (3 g, 24.6 mmol) in pyridine (180 ml) at 23 °C for 14 h. Pyridine was removed under reduced pressure and the residue was extracted with ether. The organic layer was washed with cold 3M HCl, aqueous $NaHCO_3$, and brine, and then dried and concentrated. The residue was roughly purified by silica gel (30 g; benzene) to afford (**13**) (18.4 g) as a dark red oil. This oil was treated with aqueous 46% HF (13 ml) in MeCN (184 ml) at 25 °C for 90 min after which excess of $NaHCO_3$ was added. The solvent was removed under reduced pressure and the residue was extracted with EtOAc. The organic extracts were washed, dried, and concentrated. The residue was triturated with a small amount of MeOH to give a dark solid, which was recrystallized from MeOH to afford (**14**) [6.0 g, 63% yield from (**8**)] as colourless needles; ν_{max}($CHCl_3$) 3 600 (OH), 3 450 br (OH), and 1 690 cm^{-1} (C=O); δ_H(90 MHz; $CDCl_3$) 2.73 (3 H, s, NAc), 1.7–4.2 (7 H, m), 7.1–7.9 (4 H, m, ArH). ($C_{14}H_{15}NO_2S$ requires C, 64.34; H, 5.79; N, 5.36; S, 12.27%).

(*S*)-(**14**): $[\alpha]_D^{23}$ + 13.3° (*c.* 1.14, $CHCl_3$); m.p. 158.5–160.0 °C (Found: C, 64.25, H, 5.7; N, 5.35; S, 12.35%).

(*R*)-(**14**): $[\alpha]_D^{23}$ − 14.3° (*c.* 1.20, $CHCl_3$); m.p. 160.0–161.0 °C (Found: C, 64.25; H, 5.75; N, 5.1; S, 12.4%).

4-*Azidomethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**17**). —MSA (4.4 g, 25 mmol) was added to (**14**) (5.60 g, 21 mmol) and Et_3N (6.0 ml, 43 mmol) in CH_2Cl_2 (110 ml) at −13 °C. The mixture was stirred for 10 min after which ice–water was added. The layers were separated, and the aqueous layer was extracted with ether. The combined organic layers were washed, dried, and concentrated to give (**15**) (7.0 g) as a solid.

A solution of unpurified (**15**) and NaN_3 (3.4 g, 52 mmol) in DMF (120 ml) was heated at 50 °C for 16 h. The solvent was removed under reduced pressure and the residue was extracted with ether. The combined ether layers were washed, dried, and concentrated to give the azide (**16**): ν_{max}($CHCl_3$) 2 080 (N_3) and 1 685 cm^{-1} (C=O).

Without purification, (**16**) was hydrolysed with 10% NaOH (10 ml) at 23 °C for 15 min in MeOH (40 ml)–THF (20 ml). Volatiles were removed under reduced pressure and the residue was purified by silica gel chromatography (50 g; benzene) to give (**17**) (4.0 g). Analytical HPLC (Nucleosil-$_{10}C_{18}$, 4 × 300 mm, Me_3CN–H_2O, 1:1, flow rate 2.5 ml/min) revealed that (**17**) (t_R 9.6 min) was contaminated by 2% of (**21**) (t_R 10.5). Recrystallization from MeOH afforded pure (**17**) [4.0 g, 77% yield from (**14**)]: colourless crystals; ν_{max}($CHCl_3$) 3 445 (NH) and 2 080 cm^{-1} (N_3); δ_H(90 MHz; $CDCl_3$) 2.1–2.5 (2 H, m), 2.7–4.0 (5 H, m), and 7.0–7.9 (4 H, m, ArH) ($C_{12}H_{12}N_4S$ requires C, 59.0; H, 4.95; N, 22.95; S, 13.1%).

(*S*)-(**17**): $[\alpha]_D^{23}$ + 68.4° (*c* 1.13, $CHCl_3$); m.p. 125–126 °C (Found: C, 58.85; H, 4.9; N, 22.75; S, 13.1%).

(*R*)-(**17**); $[\alpha]_D^{23}$ − 68.7° (*c* 1.26, $CHCl_3$); m.p. 125–126 °C (Found: C, 59.25; H, 4.85; N, 23.05; S, 13.0%).

4-*Aminomethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**1**). —A solution of (**17**) (3.3 g, 13.6 mmol) in ether (70 ml) was added to a suspension of $LiAlH_4$ (1.5 g, 39 mmol) in ether (30 ml) at 25 °C over a 35 min period. After being stirred for 25 min at 25 °C, the mixture was cooled to −13 °C. The reaction was quenched by successive addition of water (4.5 ml), 10% NaOH

(4.5 ml), and water (4.5 ml) again. The slurry was diluted with THF (50 ml) and stirred for 30 min at ambient temperature. The precipitate was filtered off and washed well with ether–THF. The organic layers were dried and concentrated to give (**1**) (2.80 g, 95% yield) as a colourless solid, m.p. 139–140 °C ($C_{12}H_{14}N_2S$ requires C, 66.02; H, 6.46; N, 12.83; S, 14.69%). Full details of the NMR data have been reported elsewhere.[2]

(*S*)-(**1**): $[\alpha]_D^{23}$ + 79.6 (*c* 1.18, MeOH); m.p. 141–142 °C (from EtOAc–light petroleum) (Found: C, 65.95; H, 6.4; N, 12.55; S, 14.5%).

(*R*)-(**1**): $[\alpha]_D^{23}$ − 80.1 (*c* 1.21, MeOH); m.p. 141–142.5 °C (from EtOAc–light petroleum) (Found: C, 66.15; H, 6.45; N, 12.55; S, 14.7%).

4-*Methoxycarbonylaminomethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**18**).—Methyl chloroformate (1.05 g, 11 mmol) was added dropwise at 25 °C to a solution of (**1**) (2.20 g, 10 mmol) and Et_3N (3 ml, 22 mmol) in THF (30 ml), and the mixture was stirred for 2 h at 23 °C. The volatiles were removed under reduced pressure, and the residue was extracted with ether. The organic layer was washed, dried, and concentrated. Purification of the residue by silica gel chromatography (30 g; EtOAc–benzene, 6:1) gave (**18**) (2.7 g, 97% yield) as a colourless oil; ν_{max}($CHCl_3$) 3 450 (NH), 1 720, and 1 710 cm^{-1} (C=O); $[\alpha]_D^{23}$ +21.2 (*c* 1.62, $CHCl_3$) for (*S*)-(**18**), and $[\alpha]_D^{23}$ − 22.0 (*c* 1.58, $CHCl_3$) for (*R*)-(**18**) respectively. Full details of the NMR data have been reported elsewhere.[2]

4-*Methylaminomethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]-*indole Hydrochloride* (**2**).—$LiAlH_4$ (1.2 g, 31.6 mmol) was added to a solution of (**18**) (2.7 g, 9.78 mmol) in THF (12 ml), and the resulting slurry was heated under reflux for 1 h. The mixture was worked up in a manner similar to that described for the reduction of (**17**) to (**1**) to give the free amine of (**2**) (2.1 g) as a labile viscous oil. This was immediately dissolved in a cold solution of HCl in anhydrous EtOH (5 ml). The precipitate was collected and recrystallized from MeOH to afford (**2**) [1.8 g, 65% yield from (**1**)] as colourless needles; *m/z*: 232 (M^+, 15%), 189(60), and 188 (100) ($C_{13}H_{17}ClN_2S$ requires C, 58.09; H, 6.37; N, 10.42; S, 11.93%).

(*S*)-(**2**): $[\alpha]_D^{23}$ + 68.6 (*c* 0.67, MeOH); m.p. 265–272 °C (decomp.) (Found: C, 58.0; H, 6.35; N, 10.25; S, 12.15%).

(*R*)-(**2**): $[\alpha]_D^{23}$ − 68.3 (*c* 0.74, MeOH); m.p. 265–272 °C (decomp.) (Found: C, 58.3; H, 6.4; N, 10.55; S, 12.0%).

Determination of Optical Purity of (**8**).—Compound (*R*)-(**8**) (60 mg, 0.17 mmol, $[\alpha]_D^{23}$ + 17.2°), was added to a solution of (*R*)-(+)-MTPA $[\alpha]_D^{23}$ + 69.8° (*c* 2.37, MeOH) (52 mg, 0.22 mmol), DCC (46 mg, 0.22 mmol), and DMAP (5 mg) in CH_2Cl_2 (2 ml) at 23 °C. After 2.5 h, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (7 g, benzene) to obtain (*RR*)-(**23**) as a colourless oil (90 mg, 93%). In a similar manner, (*SR*)-(**23**) was prepared from (*S*)-(**8**) ($[\alpha]_D^{23}$ − 16.8°) and (*R*)-MTPA (94% yield). The diastereoisomer composition was determined by analytical HPLC. (Waters μ-Porasil, 3.9 × 300 mm. $CHCl_3$–hexane, 1:8, flow rate 1 ml/min). The t_R values for (*RR*)-(**23**) and (*SR*)-(**23**) were 10.5 and 11.4 min, respectively.

Determination of the Optical Purity of (**11**).—Similar procedures were applied to (**11**) except that (*R*)-*O*-methyl mandelic acid[7] (m.p. 64.5–66 °C, $[\alpha]_D^{23}$ − 151.7 (*c*. 1.38, EtOH)) was used instead of MTPA. HPLC analysis: μ-Porasil, $CHCl_3$–hexane, 1:3, 2 ml/min; t_R (*SR*)-(**24**); 10.3, (*RR*)-(**24**); 11.4 min. (*SR*)-(**24**) {$[\alpha]_D^{23}$ + 55.0 (*c* 4.59, $CHCl_3$)} showed two peaks in a ratio of 96:4, indicating that (**11**) was 92% e.e. in this particular case. Thus the calculated $[\alpha]_D$ of optical pure (**11**) should be 58.0°.

Determination of Optical Purity of (**1**).—In a similar manner, both enantiomers of (**1**) were converted into the amides of (*R*)-MTPA in 98–100% yields. HPLC analysis: μ-Porasil, $CHCl_3$–hexane, 3:7, 2.0 ml/min; t_R (*SR*)-(**25**), 8.4; (*RR*)-(**25**), 9.2.

Preparations of Deuteriated Analogues of (**2**).—[10-$^2H_1$]-(**2**) was prepared from (**26**) by the method described in our earlier publication[1] and earlier sections of this paper except that $LiAlD_4$ was used instead of $LiAlH_4$. Similarly, [4-$^2H_1$]-(**2**) and [4,10-$^2H_2$]-(**2**) were prepared from [4-$^2H_1$]-(**26**).[2] Each of these isomers was obtained as an oil and was purified as the HCl salt by several recrystallizations from EtOH. These salts had no sharp m.p.s and gradually decomposed > 264 °C.

(+)-4-(4-*Bromobenzyl*)*aminomethyl*-2,3,4,9-*tetrahydrothiopyrano*[2,3-b]*indole* (**19**).—*p*-Bromobenzaldehyde (100 mg, 0.54 mmol) was added to a solution of (+)-(**1**) (100 mg, 0.46 mmol) in MeOH (2 ml), and the mixture was stirred at 23 °C. After 2 h, $NaBH_4$ (50 mg, 1.32 mmol) was added, and the mixture was stirred for an additional hour. The solvent was removed under reduced pressure and the residue was extracted with EtOAc. The organic layers were washed, dried, and concentrated. A single crystal of (**19**) was obtained from EtOH: m.p. 118–119 °C; $[\alpha]_{436}$ + 113.2 (*c* 0.454, MeOH), at 23 °C.

X-Ray Structure Determination of (**2**)·HCl *and* (**19**).—Integrated intensities were measured on a Rigaku AFC-5 diffractometer with an ω–2θ scan technique. Three standard reflections monitored every 100 reflections showed no significant change during data collection. All intensities were corrected for Lorentz and polarization factors, but not for absorption effects.

Compound (**2**)·HCl. $C_{13}H_{17}ClN_2S$, M = 268.8, monoclinic, space group $P2_1$, a = 9.199(2), b = 9.499(1), c = 8.846(1) Å, β = 116.13(1)°, Z = 2, V = 693.2(2) Å^3, D_c = 1.283 g/cm^3, radiation = Mo-K_α (λ = 0.71069 Å), μ = 4.0 cm^{-1}, crystal size = 0.3 × 0.3 × 0.3 mm, number of unique reflections = 1 302 ($2\theta_{max}$ = 50°). Crystals of pure enantiomer were obtained by recrystallization from a solution of the racemate. The structure was solved using the program MULTAN78[19] and refined by the block-diagonal least-squares method, including all the H atoms, to R = 0.033, R_W = 0.047, and S = 1.099 for 1 114 reflections with $F_o > 3\sigma(F_o)$.

Compound (**19**).—$C_{19}H_{19}BrN_2S$, M = 387.3, orthorhombic, space group $P2_12_12_1$, a = 13.051(2), b = 14.424(1), c = 9.411(1) Å, Z = 4, V = 1 771.8(4) Å^3, D_C = 1.452 g/cm^3, radiation = Cu-K_α(λ = 1.54178 Å), μ = 45.3 cm^{-1}, crystal size = 0.2 × 0.2 × 0.2 mm, number of unique reflections = 1 901 ($2\theta_{max}$ = 140°). The structure was solved by the heavy-atom method. The structure was refined by the block-diagonal least-squares method to R = 0.057, R_w = 0.076, and S = 1.056 for 1 446 reflections with $F_o > 3\sigma(F_o)$. The absolute configuration was determined by the anomalous-dispersion method, with differences between the intensities of Bijvoet pairs measured with Mo-K_α radiation (f' = −0.374, f'' = 2.456 for Br).

Pharmacological Experiments.—Experiments were performed using male albino mice (DS strain from Shionogi Farm, Japan), weighing 20–23 g (4 weeks), and male Wister (Jcl-Wister) strain rats (from Clea Japan, Inc.) weighing 180–200 g. Test compounds were dissolved in water and administered in a volume of 0.1 ml/10 g body weight of mice. These experimental details have been described elsewhere.[20]

Acknowledgements

The authors are grateful to Dr. K. Hirose and his co-workers for the biological assays, late Drs. K. Tori and J. Kikuchi for their

NMR measurements and helpful discussions. Thanks are also to T. Sato for his useful advice.

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Paper 9/01884K
Received 4*th May* 1989
Accepted 8*th August*, 1989