(s, 3 H, NCH_3), 3.84 (m, 1 H, C_4H), 4.20, 4.82 (2 t, 2 H, C_3H and C_4H), 6.36 (d, 1 H, J = 5.47 Hz, C_1H). Anal. Calcd for $C_{11}H_{13}N_4SCl$ (C, H, N, S, Cl).

7,8-Dihydro-7-methyl-8-thioxoguanosine (2). A suspension of 2-chloro-8,9-dihydro-7-methyl-9-(β-D-ribofuranosyl)-8-thioxopurin-6(1*H*)-one (**7**, 0.5 g, 1.4 mmol) in methanol (10 mL) was placed in a 200-mL stainless steel bomb and cooled in a dry ice/ethanol bath. Liquid ammonia (50 mL) was added; the bomb was sealed and heated in an oil bath (bath temperature 150 °C) overnight. The bomb was cooled in a dry ice/ethanol bath and opened. The ammonia was allowed to evaporate and the residue was chromatographed over silica gel (flash chromatography) with a mixture of ethyl acetate/acetone/water/methanol 15:1:1:1 as eluent to yield **2** as an amorphous solid (0.3 g, 60%): ^{1}H NMR (DMSO-d_6) δ 3.56 (m, 2 H, C_5H), 3.71 (s, 3 H, NCH_3), 3.78 (m, 1 H, C_4H), 4.21, 4.96 (2 dd, 2 H, C_3H and C_4H), 4.74 (t, 1 H, O*H*), 4.91 (d, 1 H, O*H*), 5.28 (d, 1 H, O*H*), 6.29 (d, 1 H, J = 5.41 Hz, C_1H), 6.62 (s, 2 H, NH_2), 11.15 (s, 1 H, N*H*); ^{13}C NMR (DMSO-d_6) δ 32.60, 62.24, 70.28, 70.42, 85.09, 89.40, 104.74, 148.75, 151.53, 153.48, 165.81. Anal. Calcd for $C_{11}H_{15}N_5O_5S$ (C, H, N, S).

Synthesis and Central Nervous System Actions of Thyrotropin-Releasing Hormone Analogues Containing a Dihydroorotic Acid Moiety†

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A series of thyrotropin-releasing hormone (TRH) analogues in which the pyroglutamic acid residue was replaced by (*S*)-4,5-dihydroorotic acid (Dio-OH) and the related derivatives were prepared. Their central nervous system actions based on spontaneous locomotor activity, antagonistic effect on reserpine-induced hypothermia, and antagonistic effect on pentobarbital anesthesia were evaluated and the structure–activity relationships are discussed. Of these, (1-methyl-(*S*)-4,5-dihydroorotyl)-L-histidyl-L-prolinamide (**14b**) showed the most potent activities, which were 30–90 times greater than those of TRH. Moreover, the thyrotropin-releasing activity of **14b** was about 50 times weaker than that of TRH, and compound 14b (TA-0910) was selected as a potent candidate.

Thyrotropin releasing hormone (TRH, **1**, L-pyroglutamyl-L-histidyl-L-prolinamide; Chart I) is known to cause a stimulating action on the central nervous system (CNS)[1] in addition to its endocrine action as a thyrotropin (TSH) releasing agent.[2] Recently, some chemical modifications of TRH to enhance the CNS actions and/or to decrease the endocrine activity have often been reported.[3-6]

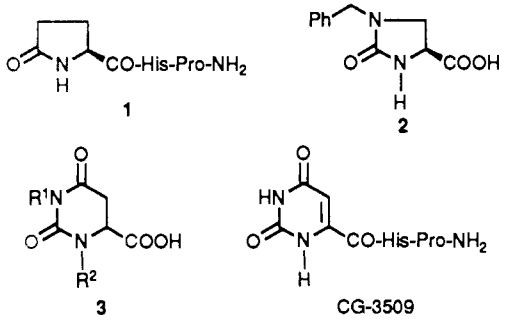
In this context, we have also studied the synthesis of TRH analogues to separate the CNS actions from the TSH-releasing activity, and through these studies we have focused on the lipophilic character in the TRH structure.[7,8] In one of our previous papers, we reported that the TRH analogue containing (*S*)-1-benzyl-2-oxoimidazolidine-4-carboxylic acid (**2**) in place of the pyroglutamic acid residue of TRH had 1.5–8 times greater CNS actions and about 16 times weaker hormonal activity than those of TRH itself.[8]

On the other hand, orotyl-His-Pro-NH_2 (CG-3509), while being clinically evaluated as a potent antidepressant, showed very weak endocrine activity (about $^1/_{13}$ times of TRH), but CNS actions were reported to be 2–3 times greater than those of TRH.[6]

Furthermore, it was reported that the analogues with six-membered rings such as L-piperidonecarboxylic acid (MK-771)[4b] and (*R*,*R*)-6-methyl-5-oxo-3-thiomorpholine-3-carboxylic acid (CG-3703)[6] in place of the pyroglutamic acid of TRH remarkably increased the CNS actions (40–66 times) on account of their complete resistance to degradation by the TRH-degrading serum enzyme and pyroglutamate aminopeptidase.[6b]

This information prompted us to attempt the enlargement of the 2-oxoimidazolidine moiety (**2**) to the related six-membered ring, and we chose the optically active dihydroorotic acid moiety, which is a hydrogenated skeleton of orotic acid (CG-3509). In this paper, we describe the syntheses and pharmacological activities of a series of TRH analogues containing various 4,5-dihydroorotic acid

Chart I



† Amino acids and their derivatives are L. Abbreviations follow the recommendations of the IUPAC–IUB Commission on Biological Nomenclature as given in *Eur. J. Biochem.* **1984**, *138*, 9–37.

‡ Research Laboratory of Applied Biochemistry.

§ Safety Research Laboratory.

(1) Plotnikoff, N. P.; Prange, A. J., Jr.; Breese, G. R.; Anderson, M. S.; Wilson, I. C. *Science*, **1972**, *178*, 417.

(2) (a) Bøler, J.; Enzmann, F.; Folkers, K.; Bowers, C. Y.; Shally, A. V. *Biochem. Biophys. Res. Commun.* **1969**, *37*, 705. (b) Burgus, R.; Dunn, T. F.; Desiderio, D.; Ward, D. N.; Vale, W.; Guillemin, R. *Nature (London)* **1970**, *226*, 321.

(3) (a) Miyamoto, M.; Fukuda, N.; Narumi, S.; Nagai, Y.; Saji, Y.; Nagawa, Y. *Life Sci.* **1981**, *28*, 861. (b) Fukuda, N.; Nishimura, O.; Shikata, M.; Hatanaka, C.; Miyamoto, M.; Saji, Y.; Nakayama, R.; Fujino, M.; Nagawa, Y. *Chem. Pharm. Bull.* **1983**, *28*, 1667.

(4) (a) Veber, D. F.; Holly, F. W.; Varga, S. L.; Hirschmann, R.; Lotti, V. J.; Porter, C. C.; Nutt, R. F. *Peptides* **1976**, *453*. (b) Nutt, R. F.; Holly, F. W.; Homnick, C.; Hirschmann, R.; Veber, D. F.; Arison, B. H. *J. Med. Chem.* **1981**, *24*, 692.

(5) (a) Brewster, D.; Dettmar, P. W.; Lynn, A. G.; Metcalf, G.; Morgan, B. A.; Rance, M. J. *Eur. J. Pharmacol.* **1980**, *66*, 65. (b) Brewster, D.; Humphrey, M. J.; Wareing, M. V. *Neuropeptides* **1981**, *1*, 153.

(6) (a) Friderichs, E.; Schwertner, E.; Herring, E.; Günzler, W. A.; Ötting, F.; Flohé, L. *Structure and Activity of Natural Peptides*; Voelter, W.; Weitzel, G., Eds.; de Gruyter: Berlin and New York, 1981; p 461. (b) Flohé, L.; Bauer, K.; Friderichs, E.; Günzler, W. A.; Hennies, H. H.; Herrling, S.; Lagler, F.; Ötting, F.; Schwertner, E. *Thyrotropin Releasing Hormone*; Griffiths, C., Bennet, G. W., Eds.; Raven Press: New York, 1983; p 327.

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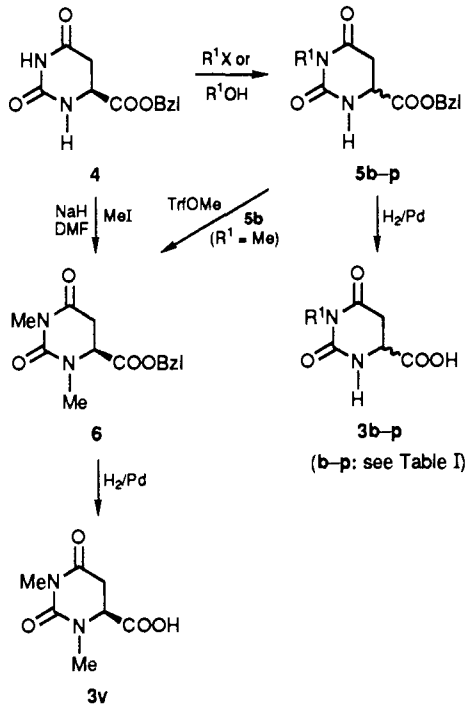
Scheme I



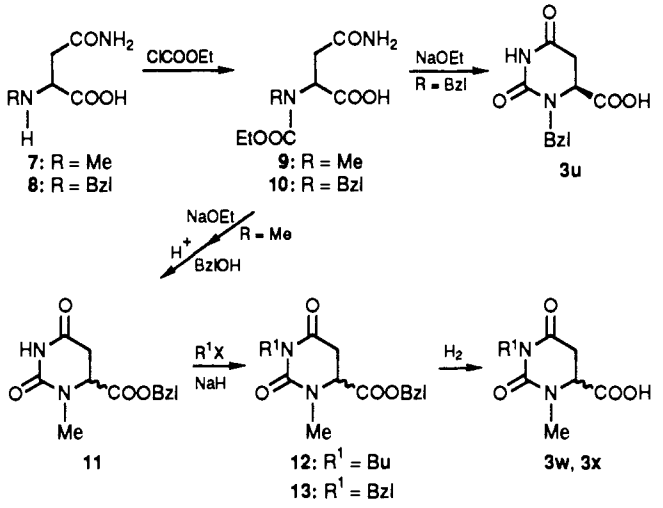
(Dio-OH, hexahydro-2,6-dioxo-4-pyrimidinecarboxylic acid) derivatives (**3**) in place of pyroglutamic acid.

Chemistry

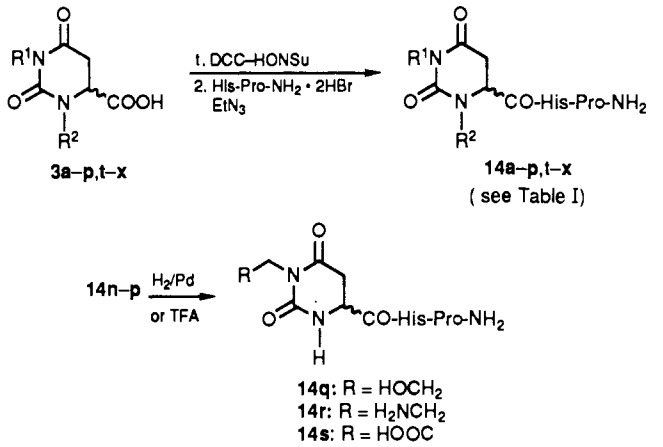
Most of the N-substituted Dio-OH (**3**) derivatives were prepared according to the methods described in our previous paper[9] as shown in Scheme I. Namely, various benzyl 1-alkyl-4,5-dihydroorotates (1-alkyl-Dio-OBzl, **5b–p**) were prepared by the reactions of optically active Dio-OBzl (**4**) with alkyl halides in the presence of sodium hydride (NaH) or potassium *tert*-butoxide (*t*-BuOK) or with alcohols in the presence of triphenylphosphine (PPh_3) and diethyl azodicarboxylate (DEAD). Then, these compounds were converted to the corresponding 1-substituted Dio-OH (**3b–p**) by catalytic hydrogenolysis. Among them, the products (**5n** and **5o**) obtained by the reactions using 2-(benzyloxy)ethyl iodide and 2-[(*tert*-butyloxycarbonyl)-(Boc)-amino]ethyl iodide in the presence of NaH did not show the optical activity because of racemization, which was confirmed by hydrogenolyses of **5n** and **5o** to give the corresponding racemic dihydroorotic acids (**3n** and **3o**).

The reaction of (*S*)-Dio-OBzl [(*S*)-**4**] with excess methyl iodide in the presence of NaH afforded 1,3-dimethyl compound **6** in 63% yield, but nuclear magnetic resonance (^1H NMR) spectroscopy using a shift reagent[10] showed that the product was partially racemized. Therefore, in order to obtain optically pure product **6**, we tried the methylation of 1-methyl-(*S*)-Dio-OBzyl (**5b**) using methyl trifluoromethanesulfonate (TrfOMe), which is a very strong methylation reagent, in the presence of NaH. Consequently, the desired product **6** having *S* configuration was obtained in a moderate yield; subsequently, the product **6** led to

Scheme II



Scheme III



1,3-dimethyl-(*S*)-Dio-OH (**3v**) by debenzylation.

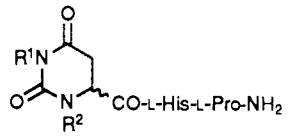
Optically active 3-benzyl-(*S*)-Dio-OH (**3u**) was obtained by ethoxycarbonylation (**10**) of L-N^{α}-benzylasparagine (**8**) followed by cyclization of **10** in the presence of sodium ethoxide (NaOEt) in ethanol. On the other hand, racemic 3-methyl-Dio-OBzl (**11**) was derived from DL-N^{α}-(ethoxycarbonyl)-N^{α}-methylasparagine (**9**) via cyclization using NaOEt followed by benzyl esterification. Moreover, N-alkylation of **11** using butyl iodide and benzyl bromide and subsequent catalytic hydrogenolysis were carried out to obtain the corresponding dihydroorotic acids **3w** and **3x** (Scheme II).

The coupling reaction of dihydroorotic acids **3a–p** with the dipeptide His-Pro-NH_2[11] was completed by a conventional dicyclohexylcarbodiimide (DCC) coupling method in the presence of *N*-hydroxysuccinimide (HONSu). The products, TRH analogues **14a–p,t–x**, were purified on a column packed with styrene–divinylbenzene copolymer resin (MCI GEL CHP-20P) by passing aqueous methanol as an eluent and were isolated as amorphous substances by lyophilization. The protecting groups of TRH analogues **14n–p** were removed by catalytic hydrogenolysis or acidolysis with trifluoroacetic acid (TFA) to obtain the corresponding tripeptides **14q–s** (Scheme III).

Furthermore, various TRH analogues **22–29**, in which Pro-NH_2 of **14b** was replaced by the other related groups, were similarly prepared by the coupling reaction of 1-

(7) Maeda, H.; Suzuki, M.; Sugano, H.; Yamamura, M.; Ishida, R. *Chem. Pharm. Bull.* **1988**, *36*, 190.
(8) Maeda, H.; Suzuki, M.; Sugano, H.; Yamamura, M.; Ishida, R. *Int. J. Pept. Protein Res.* **1989**, *33*, 403.
(9) Suzuki, M.; Maeda, H.; Kondo, K.; Sugano, H.; Matsumoto, K. *Chem. Pharm. Bull.* **1989**, *37*, 1764.
(10) Tris[3-(2,2,2-trifluoro-1-hydroxyethylidene)-*d*-camphorato]europium [Eu(TFC)$_3$, Urasol, E. Merck] (10 mg) was added to the solution of the sample (30 mg) in $CDCl_3$ (0.45 mL).
(11) Inouye, K.; Namba, K.; Otsuka, H. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1689.

Table I. Physical Properties and CNS Actions of TRH Analogues (14a–x)



14

compd	R^1	R^2	config	% yield	$[\alpha]^{23}_D$, deg (c 1, H_2O)	formula[a]	CNS actions[b] L[c] ip	CNS actions[b] R^d ip	CNS actions[b] P^e iv	toxicity and mortality[f] at 500 mg/kg iv
14a	H	H	*S*	66	−15.4	$C_{16}H_{21}N_7O_5 \cdot H_2O$	10.1	12.9	1.4	0/3
14b	Me	H	*S*	68	−13.6	$C_{17}H_{23}N_7O_5 \cdot 4H_2O$	60.6	89.4	28.1	0/3
14c	Me	H	*R*	52	−116.4	$C_{17}H_{23}N_7O_5 \cdot H_2O$	0.5	0.8	*g*	1/3
14d	Et	H	*S*	48	+14.0[h]	$C_{18}H_{25}N_7O_5 \cdot H_2O$	2.8	6.9	0.3	0/3
14e	Et	H	*R*	45	−60.6[h]	$C_{18}H_{25}N_7O_5 \cdot {}^3/_2H_2O$	0.5	0.9	0.4	0/3
14f	Pr	H	*S*	60	−20.8	$C_{19}H_{27}N_7O_5 \cdot {}^3/_2H_2O$	0.7	0.9	0.8	0/3
14g	*i*-Pr	H	*S*	71	−5.6	$C_{19}H_{27}N_7O_5 \cdot H_2O$	5.0	11.5	1.9	0/3
14h	$CH_2{=}CHCH_2$	H	*S*	59	−19.6	$C_{19}H_{27}N_7O_5 \cdot H_2O$	5.7	4.7	4.4	1/3
14i	FCH_2CH_2	H	*S*	62	−25.4	$C_{18}H_{24}FN_7O_5 \cdot H_2O$	0.9	4.1	1.2	1/3
14j	Bu	H	*S*	67	−16.0	$C_{20}H_{29}N_7O_5 \cdot H_2O$	1.2	1.8	0.8	1/3
14k	*n*-C_5H_{11}	H	*S*	65	−16.8	$C_{21}H_{31}N_7O_5 \cdot {}^3/_4H_2O$	*g*	*g*	0.4	2/3
14l	$PhCH_2$	H	*S*	67	−32.6	$C_{23}H_{27}N_7O_5 \cdot {}^3/_4H_2O$	2.3	6.8	1.4	3/3
14m	*n*-$C_{18}H_{37}$[i]	H	*S*	78	+10.0[h]	$C_{34}H_{57}N_7O_5 \cdot {}^3/_2H_2O \cdot CH_3COOH$	*g*	*g*	*g*	1/3
14n	$BzlOCH_2CH_2$	H	*RS*	70	−48.4	$C_{25}H_{31}N_7O_6$	*g*	1.1	0.4	0/3
14o	$BocNHCH_2CH_2$	H	*RS*	no isolation						
14p	*t*-$BuOCOCH_2$	H	*S*	no isolation						
14q	$HOCH_2CH_2$	H	*RS*	66	−66.4	$C_{18}H_{25}N_7O_6 \cdot 2H_2O$	*g*	*g*	*g*	0/3
14r	$H_2NCH_2CH_2$	H	*RS*	37	−52.8	$C_{18}H_{26}N_8O_5 \cdot 2H_2O$	*g*	*g*	*g*	0/3
14s	$HOOCCH_2$	H	*S*	47	−21.6	$C_{18}H_{23}N_7O_7 \cdot {}^3/_2H_2O$	*g*	0.1	0.1	0/3
14t	H	Me	*S*	22	−14.9	$C_{17}H_{23}N_7O_5 \cdot H_2O$	*g*	*g*	*g*	0/3
14u	H	$PhCH_2$	*S*	76	−67.4	$C_{23}H_{27}N_7O_5 \cdot H_2O$	0.2	*g*	0.2	1/3
14v	Me	Me	*S*	62	−16.4	$C_{18}H_{25}N_7O_5 \cdot {}^1/_2H_2O$	0.4	0.5	1.2	0/3
14w	Bu	Me	*RS*	60	−25.2[h]	$C_{21}H_{31}N_7O_5 \cdot H_2O$	*g*	*g*	*g*	0/3
14x	$PhCH_2$	Me	*RS*	59	−33.6[h]	$C_{23}H_{29}N_7O_5$	*g*	*g*	*g*	0/3

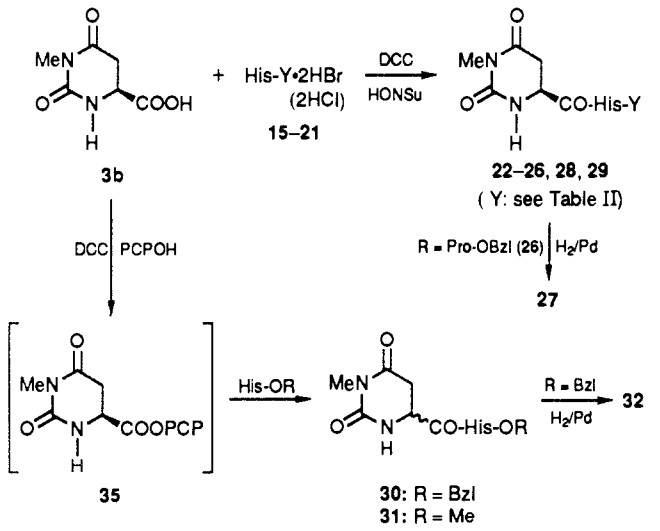
[a] All compounds exhibited satisfactory C, H, and N elemental analyses and exhibited IR and NMR spectra consistent with the structure. [b] Potency ratios of TRH analogues relative to TRH. [c] Increasing effect on spontaneous locomotor activity. [d] Antagonistic effect on reserpine-induced hypothermia. [e] Antagonistic effect on pentobarbital anesthesia. [f] Number of dead animals/number of animals used. [g] Nonactive. [h] Taken in DMF. [i] This compound was isolated as an acetate.

methyl-(*S*)-Dio-OH (**3b**) with the corresponding dipeptides (**15–21**): His-Pro-NHMe·2HBr (**15**),[12] His-Pro-NMe_2·2HBr (**16**),[13] His-Pro-OBzl·2HBr (**17**),[13] His-(*S*)-pipecolinamide (Pip-NH_2)·2HBr (**18**),[12] His-L-thiazolidine-4-carboxamide (Tzl-NH_2)·2HCl (**19**),[7] His-D-Pro-NH_2·2HBr (**20**),[14] and D-His-Pro-NH_2·2HBr (**21**).[14]

On the other hand, 1-methyl-(*S*)-Dio-His analogues **30–32**, in which Pro-NH_2 of **14b** was removed, were synthesized by the coupling reactions of pentachlorophenyl (PCP) ester **35** derived from **3b** with L-histidyl esters (His-OR). Of these, 1-methyl-(*S*)-Dio-His-OH (**32**) was obtained by catalytic hydrogenolysis of the corresponding benzyl ester compound (**30**).

Tripeptide **33**, in which the L-histidyl group of **14b** was replaced by the norvalyl (Nva) group, was prepared by the coupling reaction of **3b** with Nva-Pro-NH_2·HCl[15] in the presence of DCC and HONSu. In a similar way, the coupling reaction of 1-methylorotic acid[16] with His-Pro-NH_2 afforded 1-methylorotyl-His-Pro-NH_2 (**34**) in 54% yield (Table II). Physical data and yields of these TRH analogues are summarized in Tables I and II.

Scheme IV

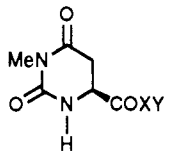


Biological Results and Discussion

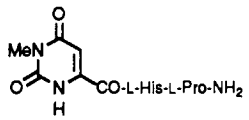
The CNS actions of the resulting TRH analogues (**14a–x** and **22–34**) were evaluated in mice by the actions on spontaneous locomotor activity, antagonistic effect on reserpine-induced hypothermia, and antagonistic effect on pentobarbital anesthesia as reported in our previous papers.[7,8] The relative potency ratio of the test compounds with respect to TRH in each CNS action was calculated by the parallel line assay method as described in the Ex-

(12) Fujino, M.; Nishimura, O.; Nagawa, Y.; Fukuda, N. Jpn. Kokai Pat. 52-116465, 1977.
(13) Tamura, T.; Iwamoto, H.; Yoshida, M.; Yamamoto, M. Jpn. Kokai Pat. 60-172996, 1985.
(14) According to the methods described in ref 11, these compounds **20** and **21** were derived from D-Pro-NH_2 and D-His as starting materials, respectively.
(15) Szirtes, T.; Kisfaludy, L.; Palosi, E.; Szporny, L. *J. Med. Chem.* **1984**, *27*, 741.
(16) Fox, J. J.; Yung, N.; Wempen, I. *Biochim. Biophys. Acta* **1957**, *23*, 295.

Table II. Physical Properties and CNS Actions of the Peptides Including the 1-Met-(*S*)-Dio Moiety



22–33

compd	X	Y	% yield	$[\alpha]^{23}_D$, deg (*c* 1, H_2O)	formula[a]	CNS actions[b] L[c] ip	R[d] ip	P[e] iv	toxicity and mortality[f] at 500 mg/kg iv
22	L-His	D-Pro-NH_2	51	+83.8	$C_{17}H_{23}N_7O_5 \cdot {}^1/_2H_2O$	*g*	*g*	*g*	0/3
23	D-His	L-Pro-NH_2	52	+19.9	$C_{17}H_{23}N_7O_5 \cdot {}^3/_4H_2O$	*g*	*g*	*g*	0/3
24	L-His	L-Pro-NHMe	65	−23.6	$C_{18}H_{25}N_7O_5 \cdot {}^5/_8H_2O$	0.4	0.9	0.6	0/3
25	L-His	L-Pro-NMe_2	55	−30.4	$C_{19}H_{27}N_7O_5 \cdot {}^5/_4H_2O$	*g*	*g*	*g*	0/3
26	L-His	L-Pro-OBzl (HCl)	81	−39.8	$C_{24}H_{28}N_6O_6 \cdot HCl \cdot H_2O$	*g*	*g*	*g*	3/3
27	L-His	L-Pro-OH	51	−17.2	$C_{17}H_{22}N_6O_5 \cdot {}^5/_4H_2O$	*g*	*g*	*g*	0/3
28	L-His	L-Pip-NH_2	69	−40.6	$C_{18}H_{25}N_7O_5 \cdot {}^1/_2H_2O$	6.7	24.9	5.0	0/3
29	L-His	L-Tzl-NH_2	47	−45.8	$C_{16}H_{21}N_7O_5S \cdot {}^3/_4H_2O$	18.8	39.8	2.1	0/3
30	L-His-OBzl		41	+21.8[h]	$C_{19}H_{21}N_5O_5$	*g*	*g*	*g*	0/3
31	L-His-OMe		31	+17.6[i]	$C_{13}H_{17}N_5O_5 \cdot 2H_2O$	*g*	*g*	*g*	0/3
32	L-His-OH		86	+68.8	$C_{12}H_{15}N_5O_5 \cdot {}^1/_4H_2O$	*g*	*g*	*g*	0/3
33	L-Nva	L-Pro-NH_2	93	−57.6	$C_{16}H_{25}N_5O_5 \cdot {}^1/_2H_2O$	*g*	*g*	*g*	0/3
34			54	−68.8	$C_{17}H_{21}N_7O_5 \cdot {}^5/_4H_2O$	*g*	*g*	0.4	0/3
CG-3509[j]						3.1	5.5	1.3	0/3
MK-771[k]						7.0	14.8	1.6	1/3

[a] All compounds exhibited satisfactory C, H, and N elemental analyses and exhibited IR and NMR spectra consistent with the structure. [b] Potecy ratios of TRH analogues relative to TRH. [c] Increasing effect on spontaneous locomotor activity. [d] Antagonistic effect on reserpine-induced hypothermia. [e] Antagonistic effect on pentobarbital anesthesia. [f] Number of dead animals/number of animals used. [g] Nonactive. [h] Taken in DMF. [i] Taken in MeOH. [j] Reference 6. [k] Reference 4b.

perimental Section, and these results are summarized in Tables I and II.

As a result, compound **14a**, in which the L-pyroglutamyl moiety was replaced by the nonsubstituted (*S*)-4,5-dihydroorotyl group, showed 10, 13, and 1.4 times greater activities, respectively, than those of TRH itself in terms of the each CNS action. It appeared that **14a** was approximately 2–3 times more potent than CG-3509 (which contains the unsaturated orotyl moiety) in stimulation of the spontaneous locomotor activity and the antagonistic effect on the reserpine-induced hypothermia.

Furthermore, the TSH-releasing activity in rats was also tested according to the known method. As listed in Table III, compound **14a** was 17 times weaker than TRH and exhibited about 1.3 times lower activity than CG-3509.[6b] From these results, it appears that **14a** is approximately 2.5–4 times superior to CG-3509 in respect to discrimination of the CNS action from the endocrine activity. Then, we investigated substituent effects on the dihydroorotyl moiety of **14a** in order to find out more effective and selective CNS-activating agents.

Of these, compound **14b**, having the methyl group at the 1-position, remarkably exhibited the activities, and the relative potency ratio to TRH was increased to about 30–90 times in each CNS action as shown in Table II. On the other hand, although the analogues having ethyl (**14d**), isopropyl (**14g**), allyl (**14h**), and benzyl (**14l**) groups at the 1-position showed more potent CNS actions than TRH, the introductions of these substituents onto the 1-position of the dihydroorotyl skeleton rather reduced the CNS actions. Compounds **14k** and **14m** substituted with the bulky groups and the derivatives **14q–s** including the hydrophilic groups showed an especially great reduction of the activities. Interestingly, **14t**, having the methyl group at the 3-position in place of the 1-position, did not show

Table III. TSH-Releasing Activity of 14a, 14b, and TRH in Rats[a]

compd	dose (iv), μg/rat	no. of rats	serum TSH (mean ± SE) at μg/100 mL	potency ratio relative to TRH[b]
control		7	44.7 ± 6.5	
14a	0.5	4	85.9 ± 25.3	0.06
	8.0	5	255.6 ± 47.9	
14b	2.0	7	132.9 ± 9.9	
	8.0	7	175.3 ± 9.3	0.02
	32.0	7	241.5 ± 21.3	
TRH	0.03125	7	59.1 ± 7.4	
	0.125	7	114.7 ± 14.4	1
	0.5	7	350.8 ± 37.6	
CG-3509				0.08[c]
MK-771				1[d]

[a] See the Experimental Section. [b] The potency ratio was calculated from the dose-response curve of TRH and sample by the parallel line assay method. [c] Reference 6b. [d] Reference 4b.

the CNS actions. Similarly, the activities of the analogues **14u–x**, having substituents at the 3-position, were very weak or disappeared.

Next, in order to investigate the roles of the prolinamide in compound **14b** showing the most potent CNS action, the related analogues **24–27** modifying the Pro-NH_2 were prepared and the CNS actions were tested. The compounds derivatized with methylamide (**24**), dimethylamide (**25**), benzyl ester (**26**), and proline (**27**) had remarkably decreased activities. Moreover, dipeptide derivatives [1-methyl-(*S*)-Dio-His analogues (**30–32**)] generated by removing the prolinamide moiety did not exhibit activity. On the other hand, the CNS actions of the analogues in which the Pro-NH_2 group was replaced by Pip-NH_2 (**28**) and Tzl-NH_2 (**29**) were considerably strong and superior to that of **14a**, but these activities did not exceed that of

Table IV. Effect on Spontaneous Locomotor Activity (SLA) of 14b and TRH in Rats[a]

compd	dose (ip), mg/kg	no. of rats	SLA for 60 min (mean ± SE)	potency ratio relative to TRH[b]
control		10	218.8 ± 33.0	
	0.1	10	241.3 ± 28.0	
14b	0.3	10	383.7 ± 42.8	39
	1.0	10	660.2 ± 58.0	
	3.0	10	894.5 ± 73.7	
control		10	219.7 ± 19.4	
	3.0	10	236.3 ± 16.1	
TRH	10.0	10	317.5 ± 38.3	1
	30.0	10	567.7 ± 78.8	

[a] See the Experimental Section. [b] The potency ratio was calculated from the dose–response curve of **14b** and TRH by the parallel line assay method.

14b. These results showed that the carboxamide might play a great role in enhancing CNS action.

From the viewpoints of conformational fit to the acceptor of TRH and resistance to enzymatic hydrolysis, the corresponding diastereomeric derivatives (**14c**, **22**, and **23**) of **14b** were also prepared. However, effective CNS actions were not observed in these compounds, and it was confirmed that the *S* configuration of the 1-methyl-4,5-dihydroorotyl moiety as well as L-His-L-Pro-NH_2 play important roles in the enhancement of the CNS activity. On the other hand, compound **33**, with the His group of **14b** replaced with a Nva group, was also investigated with the expectation of selective CNS actions;[15] however, the activities entirely disappeared. Moreover, the derivatives (**34**) introducing the methyl group at the 1-position of the orotyl moiety in CG-3509 was also synthesized in order to compare the substituent effect of the methyl group. Surprisingly, this change resulted in a remarkable decrease of the CNS actions for the compound.

Many TRH analogues containing the dihydroorotyl moiety showed stronger CNS actions than TRH, and especially, 1-methyl-(*S*)-Dio-His-Pro-NH_2 (**14b**) exhibited 30–90 times greater activities than TRH. Moreover, in order to confirm the CNS actions of compound **14b**, the effect on spontaneous locomotor activity using rats was also investigated. Consequently, **14b** exhibited 39 times greater activity than TRH as shown in Table IV. Therefore, the TSH-releasing activity of **14b** was determined in order to investigate the endocrine action. As shown in Table III, the potency was 50 times weaker than that of TRH and was $^1/_3$ that of **14a**. It was confirmed that **14b** evaluated in this present study was 1500–4500 times more selective than TRH with respect to the separation of the CNS actions from the hormonal activity. This selectivity (the CNS/endocrine ratio) might be superior to that of any other potent TRH analogues, e.g. DN-1417,[3b] MK-771,[4b] RX77368,[17] CG-3509,[6] and CG-3703,[6] which have been evaluated as efficient CNS-stimulating agents.[18]

Moreover, **14b** did not show mortality in the primitive acute toxicity test using mice after a single 500 mg/kg intravenous (iv) administration as listed in Table I and II. The LD_{50} (1590 mg/kg, iv) in mice of compound **14b** was determined by probit method using 10 male mice of ddY strain.[19]

Finally, we have selected 1-methyl-(*S*)-4,5-dihydroorotyl-L-histidyl-L-prolinamide (**14b**) as a potent candidate (TA-0910) for a therapeutic agent for CNS disorders such as consciousness disorder, senile dementia, depression. Further biological evaluation and studies of the mechanisms of the CNS actions of TA-0910 are now in progress.

Experimental Section

Melting points (mp) were measured by the use of a Yamato MP-21 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu IR-420 spectrometer. ^{1}H NMR spectra were recorded on a Hitachi R-40 (90-MHz) spectrometer using tetramethylsilane as an internal standard. Mass spectra (MS) were taken on a Hitachi M-60 spectrometer at an ionizing potential of 30 eV. Specific rotations were measured with a Perkin-Elmer 243 digital readout polarimeter using a 10-cm cell. For silica gel column chromatography, Kiesel gel 60 (0.063–0.20 mm, E. Merck) was employed for the purification of TRH analogues; MCI GEL CHP-20P (75–150 μm, Mitsubishi Chemical Industries) was utilized with aqueous MeOH as an eluent. Thin-layer chromatography (TLC) was carried out with Merck silica gel 60F-254 plates with use of the following two solvent systems: (A) BuOH–AcOH–AcOEt–H_2O (1:1:1:1) and (B) BuOH–AcOH–H_2O (4:1:1). The spots were detected under ultraviolet (UV) irradiation at 254 nm and by the use of Pauly reagent and chlorine–tolidine color sprays.

1-Substituted Dio-OBzl (5b–p). These compounds were prepared by the reaction of (*S*)-Dio-OBzl (4) with alkyl halides in the presence of base or by the Mitsunobu reaction according to the method reported by the authors.[9] The newly prepared compounds were characterized by the following data. **5i**: mp 115–117 °C; $[\alpha]^{23}_D$ +39.8° (*c* 0.5, DMF); IR (Nujol) 3250, 1720, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.65–3.2 (2 H, m), 3.95 and 4.73 (1 H each, t, *J* = 5 Hz), 4.12–4.5 (3 H, m), 5.2 (2 H, s), 6.3 (1 H, br), 7.2–7.4 (5 H, m); MS *m/z* 294 (M^+). Anal. ($C_{14}H_{15}FN_2O_4$) C, H, F, N. **5n**: mp 69–71 °C; IR (Nujol) 3250, 1725, 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.8–3.0 (2 H, m), 3.5–3.8 (2 H, m), 4.03–4.3 (3 H, m), 4.52 (2 H, s), 5.18 (2 H, s), 6.20 (1 H, br), 7.25–7.4 (10 H, m); MS *m/z* 382 (M^+). Anal. ($C_{21}H_{22}N_2O_5$) C, H, N. **5o**: mp 115–117 °C; IR (Nujol) 3300, 1740, 1720, 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (9 H, s), 2.9–3.1 (2 H, m), 3.2–3.5 (2 H, m), 3.92 (2 H, t, *J* = 4 Hz), 4.2–4.45 (1 H, m), 4.95 (1 H, br), 5.25 (2 H, s), 6.3 (1 H, br), 7.3–7.45 (5 H, m); MS *m/z* 391 (M^+). Anal. ($C_{19}H_{25}N_3O_6$) C, H, N. **5p**: mp 86–88 °C; $[\alpha]^{23}_D$ +32.6° (*c* 1, MeOH); IR (KBr) 3220, 2995, 1730, 1680 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47 (9 H, s), 2.95–3.15 (2 H, m), 4.2–4.5 (1 H, m), 4.4 (2 H, s), 5.25 (2 H, s), 6.35 (1 H, br), 7.38 (5 H, s); MS *m/z* 362 (M^+). Anal. ($C_{18}H_{22}N_2O_6$) C, H, N.

1-Substituted Dio-OH (3b–p). These compounds were prepared by catalytic hydrogenolysis of the corresponding benzyl esters (**5b–p**) in the presence of 10% Pd on charcoal according to our reported method.[9] The newly prepared compounds were characterized by the following spectral data. **3i**: mp 147–148 °C; $[\alpha]^{23}_D$ +38.6° (*c* 0.5, DMF); IR (Nujol) 3300, 1710 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.6–3.25 (2 H, m), 3.95–4.3 (3 H, m), 3.8 and 4.68 (1 H each, t, *J* = 5 Hz), 8.1 (1 H, br); MS *m/z* 204 (M^+). Anal. ($C_7H_9FN_2O_4$) C, H, F, N. **3n**: mp 104–106 °C; IR (Nujol) 3200, 1735, 1710 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.6–3.3 (2 H, m), 3.4–3.6 (2 H, m), 3.8–4.0 (2 H, m), 4.0–4.2 (1 H, m), 4.5 (2 H, s), 7.3–7.4 (5 H, m), 8.06 (1 H, br); MS *m/z* 292 (M^+). Anal. ($C_{14}H_{16}N_2O_5$) C, H, N. **3o**: mp 155–156 °C; IR (Nujol) 3300, 1735, 1720, 1680, 1630 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.45 (9 H, s), 2.8–3.0 (2 H, m), 3.1–3.5 (2 H, m), 3.91 (2 H, t, *J* = 7 Hz), 4.1–4.3 (1 H, m), 7.15 (1 H, br), 9.2 (1 H, br); MS *m/z* 301 (M^+). Anal. ($C_{12}H_{19}N_3O_6$) C, H, N. **3p** (as DCHA salt): mp 192 °C dec; $[\alpha]^{23}_D$ +4.4° (*c* 0.5, MeOH); IR (Nujol) 1720, 1690, 1635 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.41 (9 H, s), 1.0–2.2 (20 H, m), 2.6–2.9 (2 H, m), 2.8–3.2 (2 H, m), 3.5–3.8 (1 H, m), 4.2 (1 H, s), 7.31 (1 H, br). Anal. ($C_{23}H_{39}N_3O_6$) C, H, N.

Preparation of 1,3-Dimethyl-(*S*)-Dio-OBzl (6) from 5b. To a solution of 1-methyl-(*S*)-Dio-OBzl (**5b**; 1.0 g, 3.8 mmol) and TrfOMe (1.88 g, 11.4 mmol) in CH_2Cl_2 (40 mL) was added portionwise 62% NaH on paraffin (148 mg, 3.8 mmol) at 0 °C over 5 min with stirring. After stirring for 1 h at 20 °C, 10% AcOH was added to the reaction mixture to neutralize it, and the in-

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soluble solids were filtered off. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo. The residue was column chromatographed on silica gel using $CHCl_3$ as an eluent, and the crude products were crystallized from Et_2O to give **6** (400 mg, 38%): mp 65–66 °C; $[\alpha]^{23}_D$ +33.1° (*c* 1, DMF); IR (KBr) 1738, 1720, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.0–3.2 (2 H, m), 3.07 and 3.13 (3 H each, s), 4.0–4.1 (1 H, m), 5.2 (2 H, s), 7.2–7.5 (5 H, m) [No different patterns were observed in the addition of $Eu(TFC)_3$]; MS *m/z* 276 (M^+). Anal. ($C_{14}H_{16}N_2O_4$) C, H, N.

Compound 6 from 4. To a solution of (*S*)-Dio-OBzl (**4**; 1.0 g, 4 mmol) and methyl iodide (3.38 g, 24 mmol) in DMF (10 mL) was added portionwise 62% NaH on paraffin (320 mg, 8.1 mmol) at 0 °C over 15 min with stirring. After stirring for 7 h at room temperature, the reaction mixture was concentrated under reduced pressure and the residue was extracted with $CHCl_3$. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$ as an eluent to yield 0.7 g (63%): mp 54–58 °C; $[\alpha]^{23}_D$ +15.3° (*c* 1, DMF); NMR [$CDCl_3$ + $Eu(TFC)_3$] δ 4.52 and 4.6 (total 3 H, s each, ratio 3:1), 5.16 and 5.25 (total 3 H, S each, ratio 1:3).

1,3-Dimethyl-(*S*)-Dio-OH (3v). Hydrogen gas was bubbled into a mixture of benzyl ester **6** (550 mg, 2 mmol) and 10% Pd on charcoal (150 mg) in MeOH (50 mL) at room temperature for 3 h. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was recrystallized from AcOEt to afford **3v** (298 mg, 80%) as colorless prisms: mp 156–158 °C; $[\alpha]^{23}_D$ +41.2° (*c* 1, DMF); IR (Nujol) 1730, 1710, 1630 cm^{-1}; NMR (Me_2SO-d_6) δ 2.8–3.4 (2 H, m), 2.98 and 3.02 (3 H each, s), 4.2–4.4 (1 H, m). Anal. ($C_7H_{10}N_2O_4$) C, H, N.

L-N^α-Benzyl-N^α-(ethoxycarbonyl)asparagine (10). To a mixture of L-N^α-benzylasparagine (**8**; 21 g, 0.095 mol) and Et_3N (22.2 g, 0.22 mol) in H_2O (50 mL) was added dropwise ethyl chloroformate (13 g, 0.22 mol) at 5–10 °C over 15 min with vigorous stirring. After stirring for 3 h at room temperature, saturated aqueous $NaHCO_3$ (50 mL) was added to the reaction mixture and washed with AcOEt. The separated alkaline layer was adjusted to pH 2 with concentrated HCl and extracted with AcOEt (100 mL × 2). The extract was washed with H_2O, dried, and then concentrated. The residue was recrystallized from AcOEt–hexane to afford colorless prisms of **10** (6.03 g, 22%): mp 143–144 °C; $[\alpha]^{23}_D$ −105.6° (*c* 0.5, MeOH); IR (Nujol) 3350, 3200, 1720, 1695, 1655 cm^{-1}; NMR (Me_2SO-d_6) δ 1.17 (3 H, t, *J* = 8 Hz), 2.2–3.1 (2 H, m), 4.07 (2 H, q, *J* = 8 Hz), 4.48 (2 H, s), 4.6 (1 H, t, *J* = 6 Hz), 6.83 (1 H, br), 7.2–7.5 (5 H, m). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

3-Benzyl-(*S*)-Dio-OH (3u). To a mixture of sodium (1.57 g, 0.068 mol) in EtOH (150 mL) was added **10** (9.23 g, 0.03 mol) at room temperature, and the mixture was refluxed for 4 h. After evaporation of EtOH in vacuo, the remainder was diluted with H_2O and acidified with concentrated HCl and then extracted wiith AcOEt (50 mL × 2). The extract was washed with H_2O, dried, and then concentrated. The residue was crystallized from AcOEt–hexane as colorless prisms of **3u** (5.8 g, 74%): mp 178–180 °C; $[\alpha]^{23}_D$ −17.0° (*c* 1, MeOH); IR (Nujol) 3200, 1720, 1650 cm^{-1}; NMR (Me_2SO-d_6) δ 2.5–3.3 (2 H, m), 4.1 and 5.5 (2 H each, d, *J* = 15 Hz), 4.17 (1 H, d, *J* = 3 Hz), 7.32 (5 H, s). Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

3-Methyl-(*RS*)-Dio-OBzl (11). To a solution of sodium (14.5 g, 0.63 mol) in EtOH (1.74 L) was added (*RS*)-N^α-(ethoxycarbonyl)-N^α-methylasparagine[20] (**9**; 62.1 g, 0.29 mol) at room temperature and the mixture was refluxed for 2 h. After evaporation of EtOH in vacuo, Et_2O (1 L) was added to the remainder, and the insoluble products were collected by filtration to afford a crude sodium 3-methyl-(*RS*)-4,5-dihydroorotate (57.6 g, 93.5%). To a mixture of this product (21.6 g, 0.1 mol) and benzyl alcohol (75 mL, 0.67 mol) in $CHCl_3$ (100 mL) was added *p*-toluenesulfonic acid monohydrate (57 g, 0.3 mol) and the reaction mixture was refluxed for 4 h with a Dean–Stark trap to remove H_2O. When the reaction was over, $CHCl_3$ (100 mL) and H_2O (120 mL) were added to the reaction mixture. The separated organic layer was washed with saturated aqueous $NaHCO_3$, dried over $MgSO_4$, and then concentrated in vacuo. The residue was recrystallized from MeOH to afford colorless needles of **11** (21 g, 80%): mp 157–158 °C; IR (Nujol) 3200, 3100, 1730, 1685 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.0 (2 H, d, *J* = 4 Hz), 3.08 (3 H, s), 4.12 (1 H, t, *J* = 4 Hz), 5.24 (2 H, s), 7.38 (5 H, s), 8.6 (1 H, br); MS *m/z* 263 (M^+ + 1). Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

1-*n*-Butyl-3-methyl-(*RS*)-Dio-OBzl (12). To a solution of **11** (5.25 g, 0.02 mol) and *n*-butyl iodide (4.8 g, 0.026 mol) in DMF (50 mL) was added portionwise 62% NaH on paraffin (930 mg, 0.024 mol) at 25–30 °C over 10 min with stirring. After stirring overnight at room temperature, the reaction mixture was neutralized with 10% AcOH and concentrated in vacuo. The residue was extracted with AcOEt and the extract was washed with H_2O, dried over $MgSO_4$, and then evaporated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$–AcOEt (10:1) as an eluent to afford syrup of **12** (3.53 g, 58.1%): IR (film) 1740, 1715, 1675 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (3 H, d, *J* = 8 Hz), 1.0–1.6 (4 H, m), 2.98 (2 H, d, *J* = 4 Hz), 3.05 (3 H, s). 3.75 (2 H, t, *J* = 8 Hz), 4.0 (1 H, t, *J* = 4 Hz), 5.18 (2 H, s), 7.33 (5 H, s); MS *m/z* 318 (M^+).

1-Benzyl-3-methyl-(*RS*)-Dio-OBzl (13). This compound was prepared by following the procedure described for compound **12**. From 5.25 g (0.02 mol) of compound **11** and 4.45 g (0.026 mol) of benzyl bromide was obtained the title compound (2.88 g) as a syrup in 42.6% yield: IR (film) 1740, 1720, 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.0 (2 H, d, *J* = 4 Hz), 3.05 (3 H, s), 4.0 (1 H, t, *J* = 4 Hz), 4.95 (2 H, s). 5.05 and 5.10 (1 H each, d, *J* = 11 Hz), 7.1–7.5 (10 H, m); MS *m/z* 352 (M^+).

1-*n*-Butyl-3-methyl-(*RS*)-Dio-OH (3w). Hydrogen gas was bubbled into a mixture of benzyl ester **12** (3.04 g, 0.01 mol) and 10% Pd on charcoal (230 mg) in EtOH (60 mL) at room temperature for 2 h. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was dissolved in AcOEt (20 mL) and then dicyclohexylamine (DCHA; 1.8 g, 0.01 mol) was added to the solution. After the mixture was kept standing overnight in an ice box, the resulting precipitates were collected by filtration to afford **3w** (3.48 g, 85%) as the DCHA salt: mp 175–176 °C; IR (Nujol) 1700, 1660, 1640 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.9 (3 H, d, *J* = 7 Hz), 1.0–2.2 (24 H, m), 2.8–3.0 (2 H, m), 2.9 (3 H, s), 3.5–3.8 (5 H, m), 7.8 (br, s). Anal. ($C_{22}H_{39}N_3O_4$) C, H, N.

1-Benzyl-3-methyl-(*RS*)-Dio-OH (3x). This compound was prepared by following the procedure described for compound **3w**. From 3.38 g (0.01 mol) of compound **13** was obtained the title compound (3.45 g) as the DCHA salt in 78% yield: mp 173–174 °C; IR (Nujol) 1710, 1670, 1640 cm^{-1}; NMR (Me_2SO-d_6) δ 1.0–2.2 (20 H, m), 2.95 (3 H, s), 2.8–3.2 (4 H, m), 3.65–3.8 (1 H, m), 4.81 and 4.85 (1 H each, d, *J* = 15 Hz), 7.28 (5 H, s). Anal. ($C_{25}H_{37}N_3O_4$). C, H, N.

Typical Procedure for Preparation of TRH Analogues (14a–n and 14t–x). 1-Methyl-(*S*)-Dio-His-Pro-NH_2 (14b). DCC (1.4 g, 6.6 mmol) was added to a solution of 1-methyl-(*S*)-Dio-OH (**3b**; 1.03 g, 6.0 mmol) and HONSu (760 mg, 6.6 mmol) in DMF (20 mL) at 0 °C and the stirring was continued for 1.5 h. His-Pro-NH_2·2HBr[11] (2.8 g, 6.6 mmol) and Et_3N (1.4 g, 14 mmol) were added to the mixture at 0 °C, and the mixture was stirred at 10 °C for 24 h, then dicyclohexylurea (DCU) was filtered off. The filtrate was concentrated in vacuo and the residue was taken up in H_2O and the mixture was filtered again. The filtrate was adjusted to pH 8 by adding saturated aqueous $NaHCO_3$ and was passed through a column (2.7 × 34 cm) packed with MCI GEL CHP-20P. After washing the column with H_2O (300 mL), the product was eluted with 20% aqueous MeOH. The fractions containing the desired product were collected and concentrated in vacuo. The residue was lyophilized to give an amorphous substance (**14b**; 1.6 g, 68%): TLC R_f (A) 0.34, R_f (B) 0.15. This product was crystallized from a small amount of H_2O: mp 72–75 °C; $[\alpha]^{23}_D$ −13.6° (*c* 1, H_2O); IR (Nujol) 3400, 3250, 1710, 1660, 1610, 1540 cm^{-1}; ^1H NMR (D_2O) 1.6–2.3 (4 H, m), 3.0 (3 H, s), 2.7–3.2 (2 H, m), 3.6–4.0 (2 H, m), 4.2–4.6 (2 H, m), 4.7–5.2 (3 H, m), 7.0 (1 H, s), 7.71 (1 H, s). Anal. ($C_{17}H_{23}N_7O_5$·$4H_2O$) C, H, N.

In a similar way, other TRH analogues (**14a–n** and **14t–x**) were prepared, and the yields and physicochemical data are summarized in Table I.

1-(2-Hydroxyethyl)-(*RS*)-Dio-His-Pro-NH_2 (14q). Pd black (400 mg) was added to a solution of 1-[2-(benzyloxy)ethyl]-

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(*RS*)-Dio-His-Pro-NH_2 (**14n**; 1.03 g, 2 mmol) in AcOH (30 mL), and the mixture was subjected to hydrogenolysis on a Parr apparatus for 2 days at a H_2 pressure of 45 psi. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was charged on a column (3 × 35 cm) packed with MCI GEL CHP-20P, and the product was eluted with H_2O. The fractions containing the desired product were collected and concentrated in vacuo to a small volume and then lyophilized to afford **14q** (560 mg, 66%) as an amorphous substance: TLC R_f (A) 0.24, R_f (B) 0.12; IR (Nujol) 1630 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.6–2.2 (4 H, m), 2.2–3.8 (10 H, m), 4.1–4.8 (3 H, m), 6.87 and 7.50 (1 H each, s); MS *m/z* 435 (M^+).

1-(2-Aminoethyl)-(*RS*)-Dio-His-Pro-NH_2 (14r). From 1-[2-(Boc-amino)ethyl]-(*RS*)-Dio-OH (**3o**; 1.24 g, 4.1 mmol) and His-Pro-NH_2·2HBr[11] (1.78 g, 4.3 mmol) was obtained 1-[2-(Boc-amino)ethyl]-(*RS*)-Dio-Pro-NH_2 (**14o**) by elution with 50% MeOH of a column (2.6 × 28 cm) packed with MCI GEL CHP 20P by following the procedure described for compound **14b**. Subsequently, the resulting product **14o** was charged on a column of Dowex 50W × 8 (H^+ form, 50 mL, 200–400 mesh) and the column was washed with water (500 mL), and then eluted with 2% aqueous NH_3 solution. The fractions containing the desired product were collected and concentrated in vacuo. The residue was lyophilized to give **14r** (680 mg, 37%) as an amorphous substance: TLC R_f (A) 0.13, R_f (B) 0.03; IR (Nujol) 1640, 1620 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.6–2.2 (4 H, m), 2.2–4.0 (10 H, m), 4.0–4.4 (2 H, m), 4.5–4.8 (1 H, m), 6.87 and 7.41 (1 H each, br).

1-[(Hydroxycarbonyl)methyl]-(*S*)-Dio-His-Pro-NH_2 (14s). From 1-[(*tert*-butyloxycarbonyl)methyl]-(*S*)-Dio-OH (**3p**; 1.0 g, 3.68 mmol) and His-ProNH_2·2HBr[11] (1.55 g, 3.75 mmol) was obtained 1-[(*tert*-butyloxycarbonyl)methyl]-(*S*)-Dio-His-Pro-NH_2 (**14p**) by elution with 65% MeOH of a column (2.6 × 28 cm) packed with MCI GEL CHP-20P by following the procedure described for compound **14b**. The resulting product (**14p**) was charged on a column (3 × 30 cm) packed with silica gel and eluted with $CHCl_3$–MeOH (2:1). The fractions containing the desired product were collected and concentrated in vacuo. To the residue were added TFA (20 mL) and anisole (0.1 g) and the mixture was stirred for 1 h at room temperature. After evaporation of the solvent, the residue was chromatographed on silica gel using *n*-BuOH–AcOEt–AcOH–H_2O (1:1:1:1) as eluent. The crude products were charged on a column of Dowex 50W × 8 (H^+ form, 200 mL, 200–400 mesh) and washed with water (500 mL) and then eluted with 2% aqueous NH_3 solution. The solvent was concentrated in vacuo to a small volume and lyophilized to afford **14s** (818 mg, 47%) as an amorphous substance: TLC R_f (A) 0.21, R_f (B) 0.11; IR (Nujol) 1670 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.6–2.2 (4 H, m), 2.6–4.4 (10 H, m), 4.4–4.8 (1 H, m), 6.85 and 7.52 (1 H each, s).

1-Methyl-(*S*)-Dio-His Analogues (22–25, 28, and 29). These compounds were prepared by following the procedure described for compound **14b** using 1-methyl-(*S*)-Dio-OH (**3b**) and L-histidyl derivatives (**15–21**). The yields and physicochemical data are listed in Table II.

1-Methyl-(*S*)-Dio-His-Pro-OBzl Hydrochloride (26). This compound was prepared by following the procedure described for compound **14b**. From 1.56 g (9.1 mmol) of compound **3b** and 4.23 g (8.3 mmol) of His-Pro-OBzl·2HBr (**17**)[13] was obtained 1-methyl-(*S*)-Dio-His-Pro-OBzl (3.33 g) as a colorless syrup in 81% yield by elution with 70% MeOH of a column (2.6 × 28 cm) packed with MCI GEL CHP-20P. This product (650 mg) was dissolved in 1 N HCl (20 mL) and lyophilized to give the title compound **26** (690 mg, 100%) as an amorphous substance: TLC R_f (A) 0.57, R_f (B) 0.39; IR (Nujol) 1720, 1640–1680 (br) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.7–2.4 (4 H, m), 2.9 (3 H, s), 2.4–3.9 (6 H, m), 3.9–4.2 (1 H, m), 4.3–4.5 (1 H, m), 4.6–5.0 (1 H, m), 5.09 (2 H, s), 7.2–7.5 (6 H, m), 8.96 (1 H, s); MS *m/z* 496 (M^+ – HCl).

1-Methyl-(*S*)-Dio-His-Pro-OH (27). Hydrogen gas was bubbled into a mixture of 1-methyl-(*S*)-His-Pro-OBzl (**26**, 700 mg, 1.41 mmol) and Pd black (200 mg) in MeOH (20 mL) at room temperature for 3 h. H_2O (50 mL) was added to the reaction mixture to dissolve the resulting crystals and the catalyst was filtered off. The filtrate was concentrated in vacuo and the residue was crystallized with MeOH and then collected by suction to give the title compound **27** (290 mg, 51%): TLC R_f (A) 0.32, R_f (B) 0.17; mp 233–236 °C dec; IR (Nujol) 1726, 1680, 1630 cm^{-1}; NMR (D_2O) δ 1.7–2.4 (4 H, m), 2.6–3.9 (6 H, m), 3.03 (3 H, s), 4.0–4.45 (2 H, m), 4.85–5.0 (1 H, m), 7.27 (1 H, s), 8.57 (1 H, s).

1-Methyl-(*S*)-Dio-His-OBzl (30). DCC (8.3 g, 0.04 mol) was added to a solution of 1-methyl-(*S*)-Dio-OH (**3b**; 6.37 g, 0.037 mol) and pentachlorophenol (PCPOH, 11.3 g, 0.04 mol) in DMF (80 mL) at 0 °C and the stirring was continued for 1 h. His-OBzl·2TsOH (21.8 g, 0.037 mol) and Et_3N (7.47 g, 0.074 mol) were added at 0 °C, and the mixture was stirred at room temperature for 24 h, then the DCU was filtered off. The filtrate was concentrated in vacuo, and H_2O and Et_2O were added to the residue. The aqueous layer was separated and concentrated in vacuo. The residue was chromatographed on silica gel using $CHCl_3$–MeOH (4:1) as an eluent, and the crude products were recrystallized from MeOH to give **30** as colorless prisms (6.0 g, 41%): mp 194–195 °C; IR (Nujol) 3300, 3200, 1735, 1710, 1660 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.4–3.4 (4 H, m), 2.88 (3 H, s), 3.81–4.1 (1 H, m), 4.3–4.7 (1 H, m), 5.0 (2 H, s), 6.67 and 7.71 (1 H each, s), 7.1–7.3 (5 H, m), 7.72 (1 H, br); MS *m/z* 399 (M^+).

1-Methyl-(*S*)-Dio-His-OMe (31). This compound was prepared by following the procedure described for compound **30**. From 3.2 g (0.019 mol) of compound **3b** and 4.1 g (0.018 mol) of His-OMe·2HCl was obtained the title compound (1.8 g) as colorless prisms in 31% yield: mp 226–228 °C dec; IR (Nujol) 3300, 1745, 1720, 1670 cm^{-1}; ^1H NMR (Me_2SO_4-d_6) δ 2.6–3.3 (4 H, m), 2.88 (3 H, s), 3.6 (3 H, s), 3.95–4.2 (1 H, m), 4.4–4.75 (1 H, m), 7.32 and 8.9 (1 H each, s); MS *m/z* 323 (M^+).

1-Methyl-(*S*)-Dio-His-OH (32). Hydrogen gas was bubbled into a mixture of benzyl ester **30** (2.7 g, 6.8 mmol) and Pd black (50 mg) in H_2O (50 mL) at room temperature for 3 h. The catalyst was filtered off and the filtrate was concentrated in vacuo. The residue was lyophilized to give the title compound **32** (1.8 g, 86%) as an amorphous powder: TLC R_f (A) 0.30, R_f (B) 0.19; IR (Nujol) 1720, 1660 cm^{-1}; ^1H NMR (Me_2SO_4-d_6) δ 2.4–3.4 (4 H, m), 2.88 (3 H, s), 3.8–4.1 (1 H, m), 4.1–4.5 (1 H, m), 6.77 and 7.67 (1 H each, 2 s), 7.78 (1 H, br s); MS *m/z* 310 ($M^+ + 1$).

1-Methyl-(*S*)-Dio-Nva-Pro-NH_2 (33). This compound was prepared by following the procedure described for compound **14b**. From 709 mg (4.1 mmol) of compound **3b** and 1.03 g (4.12 mmol) of Nva-Pro-NH_2·HCl[15] was obtained 1.4 g (92.7%) of the title compound as an amorphous powder: TLC R_f (A) 0.63, R_f (B) 0.56; IR (Nujol) 3400, 1720, 1670 cm^{-1}; ^1H NMR (D_2O) δ 0.9 (3 H, t, J = 7 Hz), 1.2–2.5 (8 H, m), 2.9–3.2 (2 H, m), 3.1 (3 H, s), 3.6–3.9 (2 H, m), 4.2–4.7 (3 H, m); MS *m/z* 367 (M^+).

3-Methyluracilyl-6-carbonyl-His-Pro-NH_2 (34). This compound was prepared by following the procedure described for compound **14b**. From 1.0 g (5.9 mmol) of 3-methyluracil-6-carboxylic acid[16] and 2.2 g (5.3 mmol) of His-Pro-NH_2·2HBr was obtained 1.21 g (54%) of the title compound as an amorphous powder: TLC R_f (A) 0.41, R_f (B) 0.19; IR (Nujol) 1720, 1640 (br) cm^{-1}; ^1H NMR (Me_2SO_4-d_6) δ 1.6–2.2 (4 H, m), 2.7–3.8 (4 H, m), 3.1 (3 H, s), 4.1–4.3 (1 H, m), 4.55–4.9 (1 H, m), 6.2 (1 H, s), 6.9 and 7.6 (1 H each, s), 9.05 (1 H, br); MS *m/z* 403 (M^+).

CNS Activities. Effect on spontaneous locomotor activity,[3a] effect on reserpine-induced hypothermia,[3a] effect on pentobarbital anesthesia[3b] using mice were evaluated according to the same methods as described in the previous paper.[8] The potency ratio of each test compound with respect to TRH was calculated by the parallel line assay method.[21]

Effect on Spontaneous Locomotor Activity in Rats. Ten male Slc:Wistar rats (190–240 g) were used in each group. Each rat was placed in an MK-Animex meter (DSE, Muromachi Kikai) for 60 min to acclimatize them to the apparatus. The Animex meter was tuned to a sensitivity 10 μA in order to count mainly large vertical and horizontal movements, consisting of locomoter, rearing, sniffing, etc. Test compounds dissolved in physiological saline or physiological saline alone as a control were intraperitoneally (ip) administered to the rats. Spontaneous locomotor activity was measured as the total counts for 1 h after administration of a test compound. Relative potency ratio of each test compound with respect to TRH was calculated from the total counts at each dose of the test compound and TRH by the parallel line assay method.[21]

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TSH-Releasing Activity.[22] The same test as described in the previous paper[8] was employed.

Statistics. Statistical comparisons between drug-treated and control groups were performed by using two-tailed Student's *t* test.

Acute Toxicity. Three to 10 male Std/ddY mice (26–28 g) were used in each group. Test compounds dissolved in physiological saline were intravenously (iv) administered to the mice. The animals were kept under observation at 23–24 °C for 1 week, and LD_{50} was determined.[19]

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Synthesis and Antiviral Activity of Monofluoro and Difluoro Analogues of Pyrimidine Deoxyribonucleosides against Human Immunodeficiency Virus (HIV-1)

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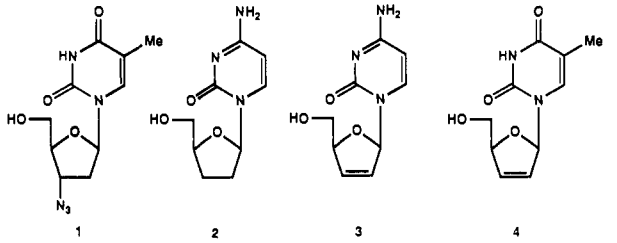
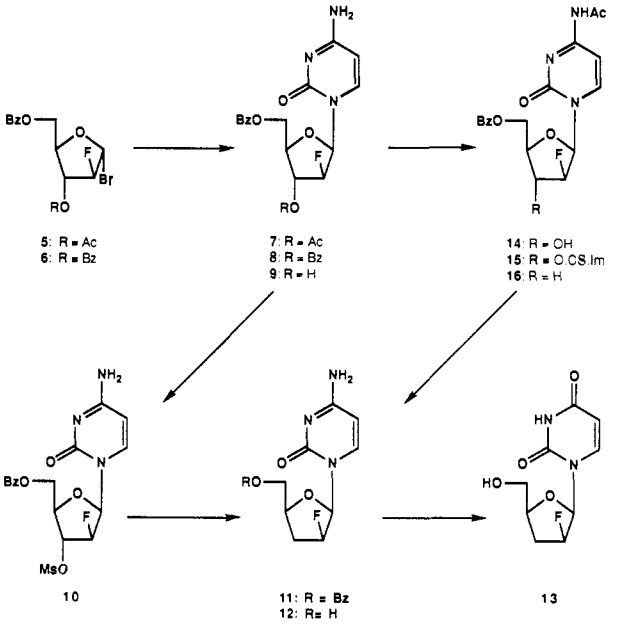
A range of 2′-fluoro and 2′,3′-difluoro analogues of pyrimidine deoxyribonucleosides have been synthesized and evaluated against human immunodeficiency virus (HIV-1) in a human lymphoblastoid cell line. Among these compounds, 1-(2,3-dideoxy-2-fluoro-β-D-threopentofuranosyl)cytosine (**12**), 2′,3′-didehydro-2′,3′-dideoxy-2′-fluorocytidine (**35**), 1-(2,3-dideoxy-2,3-difluoro-β-D-arabinofuranosyl)cytosine (**41**), and 3′-deoxy-2′,3′-didehydro-2′-fluorothymidine (**45**) were found to have significant antiviral activity, with IC_{50} values of 0.65, 10, 10, and 100 μM, respectively. The structure–activity relationships are discussed.

Since the identification of human immunodeficiency virus (HIV) as the etiological agent of acquired immunodeficiency syndrome (AIDS)[1,2] a variety of approaches have been studied in the search for an effective treatment for this disease. Inhibition of the enzyme reverse transcriptase is one approach which has been widely studied both by ourselves and others. This virally encoded enzyme is a DNA polymerase which synthesizes double-stranded DNA from the single-stranded RNA carried in virus particles. Many nucleoside derivatives have been identified which inhibit replication of this virus, particularly 2′,3′-dideoxynucleosides such as 3′-azido-3′-deoxythymidine (Zidovudine, AZT, **1**),[3] 2′,3′-dideoxycytidine (ddC, **2**),[4] 2′,3′-didehydro-2′,3′-dideoxycytidine (d4C, **3**),[5-8] and 3′-deoxy-2′,3′-didehydrothymidine (d4T, **4**).[6-10] A common feature of these compounds is the absence of a 3′-hydroxyl group in the carbohydrate moiety, and their mode of action requires metabolism to the corresponding 5′-triphosphate derivatives which act as inhibitors of reverse transcriptase and/or as chain terminators by incorporation into the growing strand of viral DNA.



1 2 3 4

Scheme I



Since the natural substrates for reverse transcriptase are 2′-deoxynucleotides, it is reasonable to suppose that both

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