Hz), 4.60 (s, 2 H), 5.10 (s, 2 H), 5.80 (d, 1 H, J = 15 Hz), 6.05–6.28 (m, 2 H), 7.23 (dd, 1 H, J = 10.5, 15 Hz), 7.33–7.40 (m, 5 H). Anal. ($C_{22}H_{31}NO_7$) C, H, N.

Ethyl 10(*S*)-[(Benzyloxycarbonyl)amino]-8,12,14-trioxapentadecanoate (25). Starting with 800 mg (1.90 mmol) of unsaturated ester **24** and using the procedure described for compound **21** gave 7.50 mg (1.76 mmol) of saturated ester **25**: ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3 H, J = 7.5 Hz), 1.30–1.65 (m, 8 H), 2.30 (t, 2 H, J = 7.5 Hz), 3.32 (s, 3 H), 3.40–3.70 (m, 6 H), 3.96 (m, 1 H), 4.12 (q, 2 H, J = 7.5 Hz), 4.62 (s, 2 H), 5.10 (s, 1 H), 5.20 (br d, 1 H), 7.35–7.40 (m, 5 H). Anal. ($C_{22}H_{35}NO_7$) C, H, N.

10(*S*)-[(Benzyloxycarbonyl)amino]-8,12,14-trioxapentadecanol (26). The ester **25** was reduced with diisobutylaluminum hydride as described in the procedure for the synthesis of alcohol **9**. Starting with 1.5 g (3.52 mmol) of ester, we obtained 1.02 g (2.66 mmol) of alcohol **26** after silica gel chromatography (EtOAc): ^{1}H NMR ($CDCl_3$) δ 1.30–1.60 (br m, 10 H), 3.32 (s, 3 H), 3.40–3.70 (m, 8 H), 3.98 (br m, 1 H), 4.60 (s, 2 H), 5.10 (s, 2 H), 7.30–7.40 (m, 5 H). Anal. ($C_{20}H_{33}NO_6$) C, H, N.

10(*S*)-[(Benzyloxycarbonyl)amino]-8,12,14-trioxapentadecanal (27). Via the procedure described for the synthesis of aldehyde **12**, alcohol **26** was oxidized to the corresponding aldehyde. Starting with 1.0 g (2.6 mmol) of alcohol, we obtained 0.90 g (2.36 mmol) of aldehyde **27** after silica gel chromatography (hexane/EtOAc, 1:1): ^{1}H NMR ($CDCl_3$) δ 1.30–1.65 (m, 8 H), 2.45 (dt, 2 H, J = 2, 7.5 Hz), 3.35 (s, 3 H), 3.40–3.70 (m, 6 H), 4.60 (s, 2 H), 5.10 (s, 2 H), 7.35–7.40 (m, 5 H). Anal. ($C_{20}H_{31}NO_6$) C, H, N.

[3*R*-[3*R,6*S**,7(*S**)]]-*N*-[*N*-[*N*-[[7-[2-[[(1,1-Dimethylethoxy)carbonyl]amino]-1-oxo-3-phenylpropyl]-6-methyl-5-oxo-1-oxa-4,7-diazacyclotetradec-3-yl]methyl]-L-valyl]-L-isoleucyl]-L-histidine Methyl Ester (6).** Using the same sequence of reactions described in detail for the synthesis of compound **4**, but replacing the aldehyde **12** with the 4-carbon homologated aldehyde **27**, provided the final cyclic compound **6**: ^{1}H NMR ($CDCl_3$) (cis Phe-Ala) δ 0.60 (d, 3 H, J = 6.6 Hz), 1.42 (s, 9 H), 3.74 (s, 3 H), 6.77 (s, 1 H), 7.58 (s, 1 H); (trans Phe-Ala) δ 1.37 (d, 3 H, J = 6.6 Hz), 1.39 (s, 9 H), 3.72 (s, 3 H), 6.78 (s, 1 H), 7.60 (s, 1 H); high-resolution FAB MS, $(M + H)^+$ 869.5496 calcd for $C_{45}H_{72}N_8O_9$ = 869.5500, meas 869.5496.

Acknowledgment. We are grateful to Dr. N. S. Andreeva for providing us with her refined atomic coordinates for porcine pepsin. We also thank Drs. R. Bott and D. R. Davies for sending us their refined atomic coordinates of the pepstatin–rhizopuspepsin complex.

Registry No. 1, 105466-99-7; **2**, 105467-06-9; **3**, 111437-53-7; **4**, 106975-94-4; **5**, 111437-54-8; **6**, 111437-55-9; **8**, 111437-56-0; **9**, 111437-57-1; **10**, 111437-58-2; 11, 111437-59-3; **12**, 111437-60-6; **13**, 111437-61-7; **14**, 111437-62-8; **15**, 111437-63-9; **16a**, 111437-64-0; **17**, 111437-65-1; **18**, 111437-66-2; **19**, 111437-67-3; **19** (debenzylated), 111437-76-4; **20**, 111437-68-4; **21**, 111437-69-5; **22**, 111437-70-8; **23**, 111437-71-9; **24**, 111437-72-0; **25**, 111437-73-1; **26**, 111437-74-2; **27**, 111437-75-3; Z-Ser-OH, 1145-80-8; H-Ala-OMe·HCl, 2491-20-5; BOC-Phe-NOS, 3674-06-4; H-Val-OBzl·HCl, 2462-34-2; H-Ile-His-Ome·HCl, 109215-25-0; $(EtO)_2P(O)CH_2CO_2Et$, 867-13-0; $(EtO)_2P(O)CH_2CH{=}CHCO_2Et$, 10236-14-3; $H_2C{=}CHCH_2Br$, 106-95-6; $MeOCH_2Cl$, 107-30-2; renin, 9015-94-5.

Analgesic Dipeptide Derivatives. 4. Linear and Cyclic Analogues of the Analgesic Compounds Arginyl-2-[(*o*-nitrophenyl)sulfenyl]tryptophan and Lysyl-2-[(*o*-nitrophenyl)sulfenyl]tryptophan

M. Teresa Garcia-López,*† Rosario González-Muñiz,† M. Teresa Molinero,‡ and Joaquin Del Rio‡

Instituto de Quimica Médica, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain, and Instituto Cajal, CSIC, Velázquez 144, 28006 Madrid, Spain. *Received May 1, 1987*

The syntheses of Trp(Nps)-Arg-OMe·HCl (**15**) [Trp(Nps) = 2-[(*o*-nitrophenyl)sulfenyl]tryptophan], its three stereoisomers, and their corresponding cyclic analogues are reported. The preparation of Trp(Nps)-Lys-OMe (**19**) and its cyclic analogue is also described. All these compounds have been designed as analogues of the analgesic dipeptide derivatives X-Trp(Nps)-OMe (**1b**, X = Arg; **2b**, X = Lys). In the case of dipeptides containing Arg or D-Arg, the coupling reactions were achieved via the isobutyl chloroformate and *N*-methylmorpholine mediated mixed anhydride procedure, while in the case of the Lys analogue, the *N*,*N*-dicyclohexylcarbodiimide method was employed. Sulfenylation reactions were carried out with Nps-Cl in acidic media. Cyclization to the diketopiperazines was achieved by using acetic acid as catalyst. The antinociceptive effects of all these new Trp(Nps)-containing dipeptides were evaluated after icv administration in mice, and the effects were compared with those of **1b**, **2b**, Tyr-Arg (Kyotorphin), and Tyr-D-Arg. The most active compounds, **15** and **19**, were found to exhibit a naloxone-reversible antinociceptive effect similar to those of **1b** and **2b** and approximately 50 and 12.5 times higher than those of Kyotorphin and its D isomer, respectively. Trp(Nps)-D-Arg-OMe·HCl, D-Trp(Nps)-Arg-OMe·HCl, and *cyclo*[Trp(Nps)-Arg]·HCl were also more effective than Kyotorphin (5, 10, and 10 times, respectively). In view of the structure–activity relationships obtained, several similarities between this series of Trp(Nps)-containing dipeptides and that of Kyotorphin analogues have emerged.

In previous papers,[1-3] it was reported that intracerebroventricular administration of the synthetic dipeptide derivatives arginyl-2-[(*o*-nitrophenyl)sulfenyl]tryptophan [Arg-Trp(Nps), **1a**], lysyl-2-[(*o*-nitrophenyl)sulfenyl]tryptophan [Lys-Trp(Nps), **2a**], and their corresponding methyl esters **1b** and **2b** shows a naloxone-reversible antinociceptive effect comparable with that of the enkephalin analogue D-Ala^2-Met-enkephalinamide (DAME). To date, two conclusions were obtained from structure–activity relationships: first, the need for a basic amino acid;[1,2] second, the importance of the Nps moiety, since no analgesia was found with the unsubstituted dipeptide Lys-Trp.[1] Concerning this moiety, the study of the analgesic effects of several Nps-modified analogues of **1** seems to

† Instituto de Quimica Médica.
‡ Instituto Cajal.

(1) Garicia-López, M. T.; Herranz, R.; González-Muñiz, R.; Naranjo, J. R.; De Ceballos, M. L.; Del Rio, J. *Peptides* **1986**, *7*, 39.
(2) Garcia-López, M. T.; González-Muñiz, R.; Herranz, R.; Molinero, M. T.; Del Rio, J. *Int. J. Pept. Protein Res.* **1987**, *29*, 613.
(3) Garcia-López, M. T.; González-Muñiz, R.; Molinero, M. T.; Naranjo, J. R.; Del Rio, J. *J. Med. Chem.* **1987**, *30*, 1658.

0022-2623/88/1831-0295$01.50/0 © 1988 American Chemical Society

Chart I

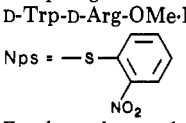
Z-Trp-Arg-OMe·HCl (3)
Z-Trp-D-Arg-OMe·HCl (4)
Z-D-Trp-Arg-OMe·HCl (5)
Z-D-Trp-D-Arg-OMe·HCl (6)
Z-Trp-Lys(Z)-OMe (7)

Trp(Nps)-Arg-OMe·HCl (15)
Trp(Nps)-D-Arg-OMe·HCl (16)
D-Trp(Nps)-Arg-OMe·HCl (17)
D-Trp(Nps)-D-Arg-OMe·HCl (18)
Trp(Nps)-Lys-OMe (19)

Z-Trp(Nps)-Arg-OMe·HCl (8)
Z-Trp(Nps)-D-Arg-OMe·HCl (9)
Z-D-Trp(Nps)-Arg-OMe·HCl (10)
Z-D-Trp(Nps)-D-Arg-OMe·HCl (11)
Z-Trp(Nps)-Lys(Z)-OMe (14)

cyclo[Trp(Nps)-Arp]·HCl (20)
cyclo[Trp(Nps)-D-Arg]·HCl (21)
cyclo[D-Trp(Nps)-Arg]·HCl (22)
cyclo[D-Trp(Nps)-D-Arg]·HCl (23)
cyclo[Trp(Nps)-Lys]·HCl (24)

D-Trp-Arg-OMe·HCl·AcOH (12)
D-Trp-D-Arg-OMe·HCl·2AcOH (13)



Z = benzyloxycarbonyl

suggest that its role is related to the adoption of a preferential active conformation in which the phenyl and the indole rings are not coplanar.[3] Studies on the mechanism of action of **2a** appear to indicate that these Trp(Nps)-containing dipeptides do not act directly on opioid receptors, but their antinociceptive effects could be possibly explained by a mixture of peptidase-inhibiting and Met-enkephalin-releasing properties.[1] Met-enkephalin-releasing action is shown by the endogenous opioid dipeptide Tyr-Arg (Kyotorphin),[4] which can be envisaged as structurally related to **1** and **2**, due to the basic (Arg or Lys) and aromatic (Tyr or Trp) nature of their constituent amino acids. This similarity is more evident when the dipeptide derivatives **1** and **2** are compared with the synthetic isomer of Kyotorphin, Arg-Tyr, which also shows analgesic effects.[5] Substitution of Arg with D-Arg in Kyotorphin or cyclization to *cyclo*(Tyr-Arg) notably increased its analgesic potency.[5,6] On the other hand, cyclic analogues of Kyotorphin in which Tyr was replaced by Trp or Trp(CHO) were almost as potent or fourfold more potent than *cyclo*(Tyr-Arg),[7] indicating that the Tyr residue can be replaced by Trp or a substituted Trp moiety. All these facts and our interest in further structure–activity relationships of these Trp(Nps)-containing dipeptides led us to prepare the isomer of the Arg derivative **1b** with reversed sequence, its three stereoisomers, and their corresponding cyclic analogues. This paper deals with the synthesis and analgesic activity of these dipeptide derivatives, including the isomer of **2b**, in which lysine is the C-terminal amino acid, and its cyclic analogue (Chart I). The antinociceptive effects of all these analogues of **1** and **2** after intracerebroventricular administration in mice are compared with those of Kyotorphin and its D-arginine analogue.

Results

Synthesis. In order to prepare Trp(Nps)-Arg-OMe·HCl (**15**), Z-tryptophan was coupled with arginine methyl ester hydrochloride, in situ generated by selective liberation of the α-NH_2 group of Arg-OMe·2HCl, via the isobutyl chloroformate and *N*-methylmorpholine mediated mixed anhydride procedure,[8] to provide Z-Trp-Arg-OMe·HCl (**3**). The other three stereoisomers of **3**, **4–6**, were similarly prepared from the corresponding D-amino acid derivatives. It is known that Trp-containing peptides react with sulfenyl halides in acidic media to give the 2-thioether derivatives.[9] Therefore, compounds **3–6** were treated with *o*-nitrobenzenesulfenyl chloride (Nps-Cl) in dry 1 N HCl in dioxane to afford the Trp(Nps) derivatives **8–11**, which on treatment with boron–tris(trifluoroacetate)/trifluoroacetic acid (BTFA/TFA), as deblocking agent for the cleavage of the Z groups,[10] gave, in the case of **8** and **9**, the desired compounds **15** and its D-Arg analogue **16**. However, in the case of the D-Trp derivatives **10** and **11**, this deprotection reaction did not lead to the expected analogues **17** and **18**, but complex mixtures of compounds, which could not be separated chromatographically, were obtained. Similar mixtures were obtained, in both cases, when trimethylsilyl iodide in acetonitrile was used as deblocking agent.[11] Although it was thought that these mixtures could be due to racemization of one or both amino acids, the comparison of their respective ^{1}H NMR spectra with those of the four stereoisomers **15–18**, after pure **17** and **18** had been obtained by an alternative route, led us to discard this possibility. This alternative route involving catalytic hydrogenolysis of **5** and **6** in ethanol containing acetic acid, using 10% Pd/C as catalyst, to remove the Z groups, followed by sulfenylation of the resulting dipeptide methyl esters **12** and **13**, gave the desired compounds **17** and **18**, respectively.

For the synthesis of Lys-containing analogue **19**, Z-Trp-Lys(Z)-OMe (**7**) was prepared by coupling Z-tryptophan with N^{ϵ}-Z-Lys-OMe by using the *N*,*N*-dicyclohexylcarbodiimide (DCC) method. Reaction of **7** with Nps-Cl in acidic medium and subsequent removal of the Z groups of the resulting Trp(Nps) derivative **14** using BTFA/TFA provided **19**.

Finally, the cyclic dipeptide derivatives **20–24** were obtained when the dipeptide esters **15–19** were refluxed in butanol containing 0.1 M acetic acid, according to the procedure described by Suzuki et al.[12] These authors have found that this acetic acid catalyzed cyclization is not subject to racemization.[6,12] Anyway, as it will be discussed below, the lack of appreciable racemization in the different steps of synthesis was easily evidenced by ^{1}H NMR spectroscopy at 300 MHz.

Structural assignments of all the compounds of this series was made on the basis of their analytical and spectroscopic data. The UV spectra of the final compounds **15–24** (Table I) showed the characteristic absorption maximum of Trp(Nps) derivatives at ~360 nm.[9] Further evidence for the presence of the Nps group in these compounds came from their ^{1}H NMR spectra, which

(4) Takagi, H.; Shiomi, H.; Ueda, H.; Amano, H. *Nature (London)* **1979**, *282*, 410.
(5) Yajima, H.; Ogawa, H.; Ueda, H.; Takagi, H. *Chem. Pharm. Bull.* **1980**, *28*, 1935.
(6) Sasaki, Y.; Akutsu, Y.; Suzuki, K.; Sakurada, S.; Kisara, K. *Chem. Pharm. Bull.* **1981**, *29*, 3403.
(7) Sasaki, Y.; Akutsu, Y.; Matsui, M.; Suzuki, K.; Sakurada, S.; Sato, T.; Kisara, K. *Chem. Pharm. Bull.* **1982**, *30*, 4435.
(8) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(9) Scoffone, E.; Fontana, A.; Rocchi, R. *Biochemistry* **1968**, *7*, 971.
(10) Pless, J.; Bauer, W. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 147.
(11) Lott, R. S.; Virander, S. C.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495.
(12) Suzuki, K.; Sasaki, Y.; Endo, N.; Mihara, Y. *Chem. Pharm. Bull.* **1981**, *29*, 233.

Table I. Physical Properties and UV Data of Linear and Cyclic Dipeptides 15–19 and 20–24

compound	no.	yield, %	mp, °C	formula[a]	UV λ_{max} (EtOH), nm (ϵ)
Trp(Nps)-Arg-OMe·HCl	15	98[b]	111[c]	$C_{24}H_{30}ClN_7O_5S$	356 (2000); 280 (9150)
Trp(Nps)-D-Arg-OMe·HCl	16	89[b]	108[c]	$C_{24}H_{30}ClN_7O_5S$	356 (2300); 281 (8550)
D-Trp(Nps)-Arg-OMe·HCl	17	94[d]	166[c]	$C_{24}H_{30}ClN_7O_5S$	356 (3500); 280 (10 400)
D-Trp(Nps)-D-Arg-OMe·HCl	18	92[d]	169[c]	$C_{24}H_{30}ClN_7O_5S$	358 (3200); 280 (10 350)
Trp(Nps)-Lys-OMe	19	61[b]	110[c]	$C_{24}H_{29}N_5O_5S\cdot 2H_2O$	356 (3150); 281 (10 400)
cyclo[Trp(Nps)-Arg]·HCl	20	77[e]	154 (dec)[f]	$C_{23}H_{26}ClN_7O_4S$	357 (2500); 280 (10 050)
cyclo[Trp(Nps)-D-Arg]·HCl	21	75[e]	158 (dec)[f]	$C_{23}H_{26}ClN_7O_4S$	358 (3200); 280 (10 200)
cyclo[D-Trp(Nps)-Arg]·HCl	22	71[e]	230 (dec)[f]	$C_{23}H_{26}ClN_7O_4S$	358 (3200); 280 (10 900)
cyclo[D-Trp(Nps)-D-Arg]·HCl	23	71[e]	136 (dec)[f]	$C_{23}H_{26}ClN_7O_4S$	358 (3200); 280 (11 300)
cyclo[Trp(Nps)-Lys]·HCl	24	86[e]	226[f]	$C_{23}H_{26}ClN_5O_4S$	357 (3400); 281 (12 700)

[a] Analytical results for C, H, Cl, N, and S were within ±0.4% of the theoretical values. [b] Yield from the corresponding Z-protected dipeptide, via method D. [c] Crystallized from *i*-PrOH/ether. [d] Yield from the corresponding 2-unsubstituted tryptophan analogue, via method C. [e] Yield from the corresponding linear dipeptide methyl ester, via method F. [f] Amorphous solid.

indicated the absence of the indole H-2 and the presence of signals attributable to this group. As in the case of the Trp(Nps)-containing dipeptides previously reported,[2,3] a significant shielding of the Nps H-6 (δ ~6.6) was observed. Comparison of the ^{1}H NMR spectra of the four linear stereoisomers **15–18** revealed that the methylene protons of arginine in the D,L and L,D dipeptides **16** and **17** are more shielded than in the L,L and D,D analogues **15** and **18**, with the γ-protons displaying the largest difference. This shielding is in agreement with various ^{1}H NMR studies of stereoisomeric dipeptides with one aromatic amino acid and the other aliphatic.[13,14] Moreover, the values of the differences of chemical shifts for the side-chain protons of arginine between **15** or **18** and **16** or **17** ($\Delta\delta_{D_2O}$ = 0.20–0.31, 0.76, and 0.21–0.27 ppm for β-, γ-, and δ-protons) are analogous to those previously reported for similar diastereomeric dipeptides containing an aliphatic side chain π-system, such as L-Phe-L-Arg and L-Phe-D-Arg ($\Delta\delta_{D_2O}$ = 0.18, 0.69, and 0.24 ppm for β-, γ-, and δ-protons).[13] Diastereomeric dipeptides of reversed sequence, i.e., L-Arg-L-Phe and L-Arg-D-Phe, have been found to exhibit similar shielding effects.[13] According to this, considerable similarity was observed between the chemical shifts of the side-chain protons of lysine in the L,L dipeptide **19** [$\delta_{Me_2SO\text{-}d_6}$ (β, γ, δ, ϵ CH_2) 1.64 and 1.65, 1.25, 1.50, and 2.73, respectively] and its reversed isomer **2b** [$\delta_{Me_2SO\text{-}d_6}$ (β, γ, δ, ϵ CH_2) 1.53, 1.33, 1.71, and 2.74, respectively]. The fact that proton chemical shifts of the aliphatic side chain in the dipeptide derivatives **15–19** depend on the absolute configuration of each amino acid residue evidenced that no racemization occurred in the different steps of synthesis.

Several differences were also found between the ^{1}H NMR spectra of the L,L or D,D cyclic dipeptides **20** or **23** and the L,D or D,L analogues **21** or **22**. Thus, the side-chain protons of arginine in **20** and **23** are more shielded than in **21** and **22**, with the β-protons, which are observably not equivalent in **20** and **23**, displaying the largest shielding effect [$\Delta\delta$ = 0.63 ppm (calculated for the more shielded β-proton)]. This significant shielding, which is also observed in the Lys derivative **24** with respect to its linear analogue **19**, is similar to that previously reported for several diketopiperazines derived from one aromatic amino acid and the other aliphatic having the same configuration.[15,16] According to previous work,[16] this fact can be attributed to the ability of the indole ring to shield cis (cyclic L,L and D,D stereoisomers **20**, **24**, and **23**) and not trans (cyclic L,D and D,L analogues **21** and **22**) methylene protons of the arginine or lysine side-chain protons. On the other hand, both amide protons in **20**, **23**, and **24** show coupling constants $J_{NH,CH}$ of ~3 Hz. However, in the L,D and D,L analogues **21** and **22**, the NH vicinal to tryptophan α-proton has J = 3.3 Hz, while the NH vicinal to arginine α-proton has J = 0 Hz. These coupling-constant values as well as the fact that the cis and trans α-protons are unequally coupled in **21** and **22** indicate a nonplanar conformation of the diketopiperazine ring.[15,17] Although in the case of **24** only one diastereomer was prepared, the fact that the ^{1}H NMR spectra of diastereomeric cyclic dipeptides are distinguishable indicates that, within the measurement limits for ^{1}H NMR, racemization of this compound did not take place.

Pharmacological Results and Discussion

The antinociceptive activity in mice of the linear and cyclic Trp(Nps)-containing dipeptides **15–19** and **20–24** given by the icv route is listed in Table II. For comparative purposes, Arg-Trp(Nps)-OMe (**1b**), Lys-Trp(Nps)-OMe (**2b**), and Kyotorphin and its isomer, Tyr-D-Arg, have also been included. As shown in this table, compounds **15** and **19** produced analgesic effects at 0.5 μg/mouse similar to those of **1b** and **2b** at the same dose, and there were no side effects. In the present assay system, a 50- and 12.5-fold higher dose of Kyotorphin and Tyr-D-Arg, respectively, was required to produce an analgesia of the same order of magnitude as that produced by **1b**, **2b**, **15**, and **19**, 5 min after injection. However, with this dose of Kyotorphin (25 μg/mouse), no effect was observed 30 min after administration. Moreover, in contrast to what happened with Kyotorphin,[5,6] substitution of Arg by D-Arg in compound **15** or cyclization of this compound resulted in less activity. Thus, Trp(Nps)-D-Arg-OMe·HCl (**16**) and *cyclo*[Trp(Nps)-Arg] (**20**) were approximately 10 and 5 times less potent than **15**, but both compounds still showed more potency than Kyotorphin. D-Trp(Nps)-Arg-OMe (**17**) was also 5 times less effective than **15**, but twice as potent as its L,D stereoisomer **16**. In all cases, the analgesia was almost completely blocked by previous administration of naloxone, 1 mg/kg sc, given 15 min before the icv injection. The close structural analogy between the compounds included in Table II and **2a**[1] suggests that the new compounds do not act directly on opioid receptors but rather, like Kyotorphin,[4] act by releasing endogenous enkephalins, or perhaps the inhibition of enkephalin-degrading enzymes also contributes to the observed pharmacological effect.[1]

No analgesia was observed with the linear D,D isomer **18** after the highest dose tested. As in the case of **2a**,[1] dose-related side effects were observed with higher doses of **16–18** and **20**. For this reason it was not considered of

(13) Deber, C. M.; Joshua, M. *Biopolymers* **1972**, *11*, 2493 and references therein.
(14) Fournié-Zaluski, M. C.; Lucas-Soroca, E.; Devin, E.; Roques, B. P. *J. Med. Chem.* **1986**, *29*, 751.
(15) Kopple, K. D.; Ohnishi, M. *J. Am. Chem. Soc.* **1969**, *91*, 962.
(16) Woodard, R. W. *J. Org. Chem.* **1985**, *50*, 4796.
(17) Bose, A. K.; Manhas, M. S.; Tavares, R. F.; Van der Ween, J. M.; Fujiwara, H. *Heterocycles* **1977**, *7*, 1227.

Table II. Analgesic Response to the Linear and Cyclic Dipeptide Derivatives **15–19** and **20–24** in the Tail-Flick Test in Mice

compound	no.	dose, μg/mouse icv	% change in reaction time (min)[a]		
			5	30	60
saline	–	–	9 ± 8	7 ± 5	5 ± 9
Trp(Nps)-Arg-OMe·HCl	**15**	0.5	113 ± 23*	63 ± 10*	20 ± 11
Trp(Nps)-D-Arg-OMe·HCl	**16**	0.5	18 ± 15	23 ± 8	9 ± 12
		2.5	20 ± 13	18 ± 10	9 ± 10
		5.0	97 ± 33*[b]	88 ± 21*[b]	30 ± 16[b]
D-Trp(Nps)-Arg-OMe·HCl	**17**	0.5	30 ± 12	26 ± 6	23 ± 9
		2.5	125 ± 28*[b]	41 ± 10*[b]	20 ± 10[b]
D-Trp(Nps)-D-Arg-OMe·HCl	**18**	0.5	6 ± 8	10 ± 5	−2 ± 3
		2.5	16 ± 4	3 ± 4	−2 ± 5
		5.0	34 ± 24[b]	42 ± 12[b]	29 ± 10[b]
Trp(Nps)-Lys-OMe	**19**	0.5	138 ± 15*	76 ± 8*	33 ± 5
cyclo[Trp(Nps)-Arg]·HCl	**20**	0.5	7 ± 12	1 ± 7	3 ± 4
		2.5	100 ± 26*[b]	60 ± 12*[b]	30 ± 11[b]
		5.0	[c]	[c]	[c]
cyclo[Trp(Nps)-D-Arg]·HCl	**21**	0.5	35 ± 16	23 ± 8	15 ± 10
cyclo[D-Trp(Nps)-Arg]·HCl	**22**	0.5	36 ± 7	31 ± 9	20 ± 6
cyclo[D-Trp(Nps)-D-Arg]·HCl	**23**	0.5	37 ± 15	22 ± 10	20 ± 7
cyclo[Trp(Nps)-Lys]·HCl	**24**	0.5	10 ± 5	12 ± 7	3 ± 4
Arg-Trp(Nps)-OMe	**1b**	0.5	120 ± 18*	52 ± 10*	22 ± 5
Lys-Trp(Nps)-OMe	**2b**	0.5	98 ± 21*	50 ± 16*	20 ± 7
Tyr-Arg (Kyotorphin)		12.5	18 ± 8	1 ± 3	6 ± 1
		25.0	98 ± 28*	31 ± 13	10 ± 9
Tyr-D-Arg		1.0	10 ± 3	5 ± 4	−2 ± 8
		3.125	79 ± 17*	34 ± 10	15 ± 9
		6.25	102 ± 12*	68 ± 11*	10 ± 4

[a] Results are the means ±SE obtained with groups of 10–12 mice. (*) Significant change ($p < 0.05$ or better, Student's t test) vs saline-treated mice. [b] Signs of weak neurotoxicity consisting in motor incoordination, respiratory disturbances, and barrel rotations. [c] Signs of strong neurotoxicity.

interest to study higher doses of the cyclic analogues **21–24**, which did not show an analgesic effect at 0.5 μg/mouse.

From the above results it can be concluded that Trp(Nps)-Arg-OMe·HCl (**15**) and Trp(Nps)-Lys-OMe·HCl (**19**) are synthetic dipeptides that, after intracerebroventricular administration, produce antinociceptive effects more marked than those produced by the endogenous opioid dipeptide Tyr-Arg (Kyotorphin). On the basis of the structural features of this series of Trp(Nps)-containing dipeptides and of the study on the mechanism of action of Lys-Trp(Nps) (**2a**),[1] we think that these dipeptide derivatives can be considered as related to Kyotorphin. Further support for this contention emerges when certain structure–activity relationships, obtained in this series and in that of Kyotorphin analogues, are compared. This comparison reveals the following similarities: (a) The basicity of the aliphatic amino acid is very important for activity.[1,7] (b) The amino acid sequence can be reversed without affecting significantly the effect.[5] (c) The activity depends on the absolute configuration of each amino acid residue.[5,6] (d) Cyclization to the corresponding 2,5-diketopiperazine derivatives leads to changes in the potency.[6] However, the absolute configuration of the amino acids and the cyclization affects differentially the antinociceptive activity of the compounds herein described and of those related to Kyotorphin. (e) The presence of substituents on the aromatic ring of the aromatic amino acid and the nature of these substituents strongly affect the activity.[1,3,7] Furthermore, the pharmacological results obtained suggest that the Nps group at position 2 of tryptophan is a suitable substituent to produce a marked analgesic activity. It would probably be of interest to search for new modified analogues of these Trp(Nps)-containing dipeptides with longer-lasting antinociceptive activity and less pronounced side effects.

Experimental Section

Chemical Methods. Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 or a Varian XL-300 spectrometer operating at 90 or 300 MHz, respectively, using Me_4Si as internal standard. UV absorption spectra were taken with a Perkin-Elmer 550 SE spectrophotometer. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel $60F_{254}$ (Merck). Silica gel 60 (230–400 mesh) (Merck) was used for column chromatography. Compounds were detected with UV light (254 nm).

All the amino acids used were of the L configuration unless otherwise specified. N^{α}-(Benzyloxycarbonyl)tryptophan (L and D configurations) was synthesized as described in the literature.[18,19] Arginine methyl ester dihydrochloride (L and D configurations) and N^{ϵ}-(benzyloxycarbonyl)lysine methyl ester hydrochloride were prepared as described.[20–22] Tyr-Arg (Kyotorphin) and Tyr-D-Arg were purchased from Bachem. *o*-Nitrobenzenesulfenyl chloride was from Sigma.

General Procedures. Method A. Coupling Reaction with Isobutyl Chloroformate/*N*-Methylmorpholine (Mixed Anhydride Method). To a solution of the N^{α}-(benzyloxycarbonyl)tryptophan (5.0 mmol) in dry THF (12 mL) were added at −20 °C *N*-methylmorpholine (5.9 mmol) and isobutyl chloroformate (5.9 mmol). The mixture was stirred at that temperature for 15 min, followed by the addition of a solution of the arginine methyl ester dihydrochloride (5.9 mmol) and $NaCO_3H$ (5.9 mmol) in H_2O (6 mL) at 0 °C. Stirring was continued at that temperature for 1 h. Solvents were removed under reduced pressure, and the residue was chromatographed on a silica gel column with $CHCl_3$/MeOH (6:1).

Method B. *o*-Nitrophenylsulfenylation Reactions in 1 N HCl/Dioxane. To a solution of Z protected dipeptide methyl ester or Z protected dipeptide methyl ester hydrochloride (2 mmol) in 1 N HCl/dioxane (15 mL) was added Nps-Cl (3 mmol), and the mixture was stirred at room temperature for 30 min. Removal of the solvent left a residue, which was purified on a silica gel column using $CHCl_3$/MeOH (9:1).

(18) Smith, E. L. *J. Biol. Chem.* **1948**, *175*, 39.
(19) Yahima, H.; Kubo, K. *J. Am. Chem. Soc.* **1965**, *87*, 2039.
(20) De Tar, D. F.; Rogers, F. F.; Bach, H. *J. Am. Chem. Soc.* **1967**, *89*, 3039.
(21) Greenstein, J. P.; Winitz, M.; Gullino, P.; Birnbaun, S. M.; Otey, M. C. *Arch. Biochem. Biophys.* **1956**, *64*, 342.
(22) Slotin, L. A.; Lauren, D. R.; Williams, R. E. *Can. J. Chem.* **1977**, *55*, 4257.

Method C. *o*-Nitrophenylsulfenylation Reactions in Acetic Acid. To a solution of dipeptide methyl ester hydrochloride (1 mmol) in AcOH (5 mL) was added Nps-Cl (1.1 mmol), and the mixture was stirred at room temperature for 1 h. Dry diethyl ether (20 mL) was added, and the solid obtained was filtered and purified on a silica gel column using $CHCl_3$/MeOH (6:1). Yields, physical characteristics, and UV data of compounds 17 and 18, obtained from 12 and 13, respectively, by this method, are recorded in Table I. ^1H NMR data are listed in the supplementary material.

Method D. Removal of the Benzyloxycarbonyl Protecting Group with Boron–Tris(trifluoroacetate)/Trifluoroacetic Acid. To a cooled solution (0 °C) of Z protected dipeptide methyl ester hydrochloride (1.5 mmol) in TFA (2.5 mL) was added BTFA in TFA (3 equiv), and the mixture was stirred at room temperature for 20 h. Removal of the solvent and purification of the residue on a silica gel column using $CHCl_3$/MeOH (9:1) gave the unprotected dipeptides. Yields, physical characteristics, and UV data of compounds 15, 16, and 19, obtained from 8, 9, and 14, respectively, by this method, are recorded in Table I. ^1H NMR data are recorded in the supplementary material.

Method E. Removal of the Benzyloxycarbonyl Protecting Group by Hydrogenolysis. A solution of Z protected dipeptide methyl ester (1.5 mmol) in a mixture of EtOH (80 mL) and AcOH (1.5 mL) was hydrogenated at 20 psi and room temperature in the presence of 10% Pd/C (0.85 g) for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated to leave the crude product as a chromatographically homogeneous syrup which needed no further purification.

Method F. Preparation of 2,5-Diketopiperazines. Dipeptide methyl ester hydrochloride (0.8 mmol) was dissolved in 0.1 M AcOH/*n*-BuOH (10 mL), and the solution was refluxed for 4 h. Solvents were removed under reduced pressure, and the crude product was purified on a silica gel column using $CHCl_3$/MeOH (6:1). Yields, physical characteristics, and UV data of the cyclic dipeptides **20–24**, obtained from 15–19, respectively, by this method, are listed in Table I. ^1H NMR data are listed in the supplementary material.

N^{α}-(Benzyloxycarbonyl)tryptophanylarginine Methyl Ester Hydrochloride (3). The title compound was prepared by coupling Z-Trp-OH with Arg-OMe·HCl according to method A: yield 90% (recrystallized from acetone); mp 163–164 °C; ^1H NMR (Me_2SO-d_6) δ 1.30–1.90 (m, 4 H, Arg β- and γ-CH_2), 3.10 (m, 4 H, Trp β- and Arg δ-CH_2), 3.63 (s, 3 H, methyl), 4.33 (m, 2 H, Trp α- and Arg α-CH), 4.95 (s, 2 H, benzyl CH_2), 7.30 (s, 5 H, benzyl C_6H_5). Anal. ($C_{26}H_{33}ClN_6O_5$) C, H, Cl, N.

N^{α}-(Benzyloxycarbonyl)tryptophanyl-D-arginine Methyl Ester Hydrochloride (4). The title compound was prepared by coupling Z-Trp-OH with D-Arg-OMe·HCl according to method A: yield 88% (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.20–2.00 (m, 4 H, Arg β- and γ-CH_2), 3.10 (m, 4 H, Trp β- and Arg δ-CH_2), 3.65 (s, 3 H, methyl), 4.33 (m, 2 H, Trp α- and Arg α-CH), 4.97 (s, 2 H, benzyl CH_2), 7.30 (s, 5 H, benzyl C_6H_5). Anal. ($C_{26}H_{33}ClN_6O_5$) C, H, Cl, N.

N^{α}-(Benzyloxycarbonyl)-D-tryptophanylarginine Methyl Ester Hydrochloride (5). This compound was prepared by coupling Z-D-Trp-OH with Arg-OMe·HCl according to method A: yield 68% (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.10–1.90 (m, 4 H, Arg β- and γ-CH_2), 3.05 (m, 4 H, Trp β- and Arg δ-CH_2), 3.61 (s, 3 H, methyl), 4.30 (m, 2 H, Trp α- and Arg α-CH), 4.93 (s, 2 H, benzyl CH_2), 7.30 (s, 5 H, benzyl C_6H_5). Anal. ($C_{26}H_{33}ClN_6O_5$) C, H, Cl, N.

N^{α}-(Benzyloxycarbonyl)-D-tryptophanyl-D-arginine Methyl Ester Hydrochloride (6). This compound was prepared by coupling Z-D-Trp-OH with D-Arg-OMe·HCl according to method A: yield 89% (recrystallized from acetone); mp 168–169 °C; ^1H NMR (Me_2SO-d_6) δ 1.30–2.00 (m, 4 H, Arg β- and γ-CH_2), 3.20 (m, 4 H, Trp β- and Arg δ-CH_2), 3.70 (s, 3 H, methyl), 4.25 (m, 2 H, Trp α- and Arg α-CH), 5.00 (s, 2 H, benzyl CH_2), 7.37 (s, 5 H, benzyl C_6H_5). Anal. ($C_{26}H_{33}ClN_6O_5$) C, H, Cl, N.

N^{α}-(Benzyloxycarbonyl)tryptophanyl-N^{ϵ}-(benzyloxycarbonyl)lysine Methyl Ester (7). To a stirred solution of Z-Trp-OH (2.36 g, 7 mmol) in dry THF (20 mL) were added triethylamine (1 mL, 7 mmol) and N^{ϵ}-Z-Lys-OMe·HCl (2.32 g, 7 mmol), and stirring was continued at room temperature for 15 min. Then, DCC (1.44 g, 7 mmol) was added and the reaction mixture was stirred for an additional 2 h. The urea formed was removed by filtration, and the filtrate was evaporated to dryness. Recrystallization of the product from EtOAc/hexane gave 3.8 g (91%) of 7: mp 89–91 °C; ^1H NMR (Me_2SO-d_6) δ 3.07 (m, 4 H, Trp β- and Lys ϵ-CH_2), 3.62 (s, 3 H, methyl), 4.32 (m, 2 H, Trp α- and Lys α-CH), 4.95 and 5.00 (2 s, 4 H, 2 benzyl CH_2), 7.32 (s, 10 H, 2 benzyl C_6H_5). Anal. ($C_{34}H_{38}N_4O_7$) C, H, N.

$N\alpha$-(Benzyloxycarbonyl)-2-[(*o*-nitrophenyl)sulfenyl]tryptophanylarginine Methyl Ester Hydrochloride (8). The title compound was prepared from 3 according to method B: yield 90%; mp 103–104 °C (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.30–1.80 (m, 4 H, Arg β- and γ-CH_2), 3.15 (m, 4 H, Trp β- and Arg δ-CH_2), 3.56 (s, 3 H, methyl), 4.35 (m, 2 H, Trp α- and Arg α-CH), 4.90 (s, 2 H, benzyl CH_2), 6.66 (dd, 1 H, Nps H-6), 7.27 (m, 5 H, benzyl C_6H_5), 8.27 (dd, 1 H, Nps H-3). Anal. ($C_{32}H_{36}ClN_7O_7S$) C, H, Cl, N, S.

N^{α}-(Benzyloxycarbonyl)-2-[(*o*-nitrophenyl)sulfenyl]tryptophanyl-D-arginine Methyl Ester Hydrochloride (9). This compound was prepared from 4 according to method B: yield 75%; mp 113–114 °C (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.10–1.85 (m, 4 H, Arg β- and γ-CH_2), 3.07 (m, 4 H, Trp β- and Arg δ-CH_2), 3.61 (s, 3 H, methyl), 4.10 (m, 1 H, Trp α-CH), 4.47 (m, 1 H, Arg α-CH), 4.90 (s, 2 H, benzyl CH_2), 6.67 (dd, 1 H, Nps H-6), 7.30 (m, 5 H, benzyl C_6H_5), 8.27 (dd, 1 H, Nps H-3). Anal. ($C_{32}H_{36}ClN_7O_7S$) C, H, Cl, N, S.

N^{α}-(Benzyloxycarbonyl)-2-[(*o*-nitrophenyl)sulfenyl]-D-tryptophanylarginine Methyl Ester Hydrochloride (10). This compound was prepared from 5 according to method B: yield 80%; mp 135–136 °C (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.10–1.80 (m, 4 H, Arg β- and γ-CH_2), 2.93 (m, 2 H, Arg δ-CH_2), 3.13 (m, 2 H, Trp β-CH_2), 3.56 (s, 3 H, methyl), 4.00 (m, 1 H, Trp α-CH), 4.43 (m, 1 H, Arg α-CH), 4.90 (s, 2 H, benzyl CH_2), 6.63 (dd, 1 H, Nps H-6), 7.25 (m, 5 H, benzyl C_6H_5), 8.25 (dd, 1 H, Nps H-3). Anal. ($C_{32}H_{36}ClN_7O_7S$) C, H, Cl, N, S.

N^{α}-(Benzyloxycarbonyl)-2-[(*o*-nitrophenyl)sulfenyl]-D-tryptophanyl-D-arginine Methyl Ester Hydrochloride (11). This compound was prepared from 6 according to method B: yield 89%; mp 95–96 °C (amorphous solid); ^1H NMR (Me_2SO-d_6) δ 1.30–2.00 (m, 4 H, Arg β- and γ-CH_2), 3.20 (m, 4 H, Trp β- and Arg δ-CH_2), 3.57 (s, 3 H, methyl), 4.40 (m, 2 H, Trp α- and Arg α-CH), 4.90 (s, 2 H, benzyl CH_2), 6.70 (dd, 1 H, Nps, H-6), 7.30 (m, 5 H, benzyl C_6H_5), 8.30 (dd, 1 H, Nps H-3). Anal. ($C_{32}H_{36}ClN_7O_7S$) C, H, Cl, N, S.

D-Tryptophanylarginine Methyl Ester Hydrochloride Acetate (12). The title compound was obtained from 5 according to method E: yield 89%; homogeneous syrup; ^1H NMR (Me_2SO-d_6) δ 1.15–1.80 (m, 4 H, Arg β- and γ-CH_2), 1.88 (s, 3 H, acetate OOCCH_3), 3.05 (m, 4 H, Trp β- and Arg δ-CH_2), 3.63 (s, 3 H, methyl), 4.16 (m, 1 H, Arg α-CH), 7.20 (d, 1 H, indole H-2). No attempts to obtain analytical data of this hygroscopic compound were made.

D-Tryptophanyl-D-arginine Methyl Ester Hydrochloride Acetate (13). This compound was obtained from 6 according to method E: yield 91%; homogeneous syrup; ^1H NMR (Me_2SO-d_6) δ 1.40–1.90 (m, 4 H, Arg β- and δ-CH_2), 1.86 (s, 6 H, 2 acetate OOCCH_3), 3.10 (m, 4 H, Trp β- and Arg δ-CH_2), 3.60 (s, 3 H, methyl), 4.30 (m, 1 H, Arg α-CH), 7.20 (d, 1 H, indole H-2). No attempts to obtain analytical data of this hygroscopic compound were made.

N^{α}-(Benzyloxycarbonyl)-2-[(*o*-nitrophenyl)sulfenyl]tryptophanyl-N^{ϵ}-(benzyloxycarbonyl)lysine Methyl Ester (14). The title compound was prepared from 7 according to method B: yield 82%; mp 70–71 °C (amorphous solid); ^1H NMR ($CDCl_3$) δ 3.00 (m, 2 H, Lys ϵ-CH_2), 3.30 (d, 2 H, Trp β-CH_2), 3.61 (s, 3 H, methyl), 4.45 (m, 2 H, Trp α- and Lys α-CH), 5.00 (s, 4 H, 2 benzyl CH_2), 6.63 (dd, 1 H, Nps H-6), 7.26 (s, 10 H, 2 benzyl C_6H_5), 8.16 (dd, 1 H, Nps H-3). Anal. ($C_{40}H_{40}N_5O_9S$) C, H, N, S.

Analgesia Assay. Analgesia was evaluated in male ICR Swiss albino mice weighing 20–25 g by means of the tail-flick test carried out in the manner described by Nott[23] with a cutoff time of 10 s. The pain reaction was recorded 30 min before the administration of any drug or saline and at various times later. The control

(23) Nott, M. V. *Eur. J. Pharmacol.* **1968**, *5*, 93.

reaction time was in the range of 1.8–2 s. The peptides were dissolved in 0.01 N HCl, neutralized with 0.1 M NaOH, and injected intracerebroventricularly into conscious animals at a constant volume of 5 μL. The Student's t test was used for statistical comparisons.

Acknowledgment. We thank the Consejo Superior de Investigaciones Cientificas and the Comisiôn Asesora de Investigaciôn Cientifica y Tecnica for financial support.

Registry No. 3, 111237-79-7; 4, 111237-80-0; 5, 111237-81-1; 6, 111237-82-2; 7, 111237-83-3; 8, 111237-84-4; 9, 111237-85-5; 10, 111237-86-6; 11, 111237-87-7; 12, 111237-89-9; 13, 111237-90-2; 14, 111237-91-3; 15, 111237-92-4; 15 (free base), 111238-00-7; 16, 111237-93-5; 16 (free base), 111238-01-8; 17, 111237-94-6; 17 (free base), 111238-02-9; 18, 111237-95-7; 18 (free base), 111238-03-0; 19, 111237-96-8; 20, 111237-97-9; 20 (free base), 111320-54-8; 21, 111320-51-5; 21 (free base), 111407-15-9; 22, 111320-52-6; 22 (free base), 111407-16-0; 23, 111320-53-7; 23 (free base), 111407-17-1; 24, 111237-98-0; 24 (free base), 111320-55-9; Z-Trp-OH, 7432-21-5; D-Arg-OMe·HCl, 111237-99-1; Z-D-Trp-OH, 2279-15-4; Arg-OMe·HCl, 18598-71-5; N^{ϵ}-Z-Lys-OMe·HCl, 27894-50-4; Nps-Cl, 7669-54-7.

Supplementary Material Available: Tables of ^{1}H NMR data containing proton chemical shifts of 15–24 and coupling constants of amide protons in 20–24 (2 pages). Ordering information is given on any current masthead page.

Crystal Structures and Pharmacologic Activities of 1,4-Dihydropyridine Calcium Channel Antagonists of the Isobutyl Methyl 2,6-Dimethyl-4-(substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylate (Nisoldipine) Series

R. Fossheim,*† A. Joslyn,‡,⊥ A. J. Solo,§ E. Luchowski,‡ A. Rutledge,‡ and D. J. Triggle*‡

Department of Chemistry, University of Oslo, Blindern 0315, Oslo 3, Norway, and Departments of Biochemical Pharmacology and Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.
Received March 11, 1987

A series of isobutyl methyl 2,6-dimethyl-4-(X-substituted phenyl)-1,4-dihydropyridine-3,5-dicarboxylates (X = H, 2-NO_2, 3-NO_2, 3-CN, 3-MeO, 4-F, 2-CF_3, 3-CF_3, and 4-Cl) related to and including nisoldipine (X = 2-NO_2) has been synthesized, their solid-state structures determined by X-ray analysis (X = H, 2-NO_2, 3-NO_2, 3-CN, 3-MeO, and 4-F), and their pharmacologic activities determined, as the racemic compounds, against [^{3}H]nitrendipine binding and K^+-depolarization-induced tension responses in intestinal smooth muscle as measures of Ca^{2+} channel antagonist activity. Comparisons of structure are presented to previously analyzed 1,4-dihydropyridines. The degree of 1,4-dihydropyridine ring puckering is dependent on the nature and position of the phenyl ring substituent and the adopted interring conformation. Different ester substituents affect 1,4-dihydropyridine ring puckering to a small extent in most cases. Pharmacologic and radioligand binding activities for the nine compounds studied show a parallel dependence on phenyl ring substituent, but the compounds are approximately 10-fold more active in the radioligand binding assay than in the pharmacologic assay. Consistent with a previous report for the nifedipine series (Fossheim et al. *J. Med. Chem.* **1982**, *25*, 126), pharmacologic activity increases with increasing 1,4-dihydropyridine ring planarity.

The Ca^{2+} channel antagonists are a heterogeneous group of agents possessed of a common property, the ability to block current through potential-dependent Ca^{2+} channels.[1-4] Verapamil, diltiazem, and nifedipine, as representatives of the phenylalkylamine, benzothiazepine, and 1,4-dihydropyridine categories of Ca^{2+} channel drugs, enjoy substantial use in cardiovascular medicine.[2,5,6] Because of their potent and generally selective activities, these drugs are of increasing prominence as molecular tools with which to probe Ca^{2+} channel structure and function.[6-8]

The 1,4-dihydropyridines have assumed particular importance in the analysis of Ca^{2+} channel structure and function because of the availability of many analogues of nifedipine and the existence of both activator and antagonist ligands. Pharmacologic and radioligand binding studies are consistent with actions at specific membrane sites. Published structure–activity correlations indicate that the nature and position of the substituent in the aryl ring of the 1,4-dihydropyridine antagonists are very important determinants of the antagonist activity, which increases with minimum Sterimol width of the ortho or meta substituent and with the electron-withdrawing capacity of the meta substituent.[3,6,9-12] Our previous X-ray structural studies of phenyl ring substituted analogues of nifedipine indicate that the degree of puckering of the 1,4-dihydropyridine ring is related to pharmacologic activity, the latter increasing with increasing ring planarity.[13,14] However, only a limited and homogeneous series

† University of Oslo.
‡ Department of Biochemical Pharmacology, School of Pharmacy, State University of New York at Buffalo.
§ Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo.
⊥ Present address: Smith Kline and French Laboratories, Swedeland, PA.

(1) Fleckenstein, A. *Annu. Rev. Pharmacol. Toxicol.* **1977**, *17*, 149.
(2) Fleckenstein, A. *Calcium Antagonism in Heart and Smooth Muscle. Experimental Facts and Therapeutic Prospects*; Wiley-Interscience: New York, NY, 1983.
(3) Janis, R. A.; Triggle, D. J. *J. Med. Chem.* **1985**, *26*, 775.
(4) Triggle, D. J.; Janis, R. A. *Annu. Rev. Pharmacol. Toxicol.* **1987**, *27*, 347.
(5) Bühler, F. R., Distler, A., Gross, F., Eds. *J. Cardiovas. Pharmacol.* **1984**, *6*, Suppl. 7.
(6) Janis, R. A.; Triggle, D. J. *Drug Dev. Res.* **1984**, *4*, 257.
(7) Glossmann, H.; Ferry, D. R.; Goll, A.; Streissnig, J.; Zernig, G. *Arzneim.-Forsch.* **1985**, *35*, 1917.
(8) Hess, P.; Fox, A. P.; Lansman, J. B.; Nilius, B.; Nowycky, M. C.; Tsien, R. W. *Ion Channels in Neural Membranes*; Ritchie, J. M., Keynes, R. D., Bolis, L., Eds.; Alan R. Liss: New York, 1986; pp 227–252.
(9) Loev, B.; Goodman, M. M.; Snader, K. M.; Tedeschi, R.; Macko, E. *J. Med. Chem.* **1974**, *17*, 956.
(10) Rodenkirchen, R.; Bayer, R.; Steiner, R.; Bossert, F.; Mayer, H.; Moller, E. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1978**, *310*, 69.
(11) Bolger, G. T.; Gengo, P.; Klockowski, R.; Luchowski, E.; Siegel, H.; Janis, R. A.; Triggle, A. M.; Triggle, D. J. *J. Pharmacol. Exptl. Therap.* **1983**, *225*, 291.
(12) Mahmoudian, M.; Richards, G. W. *J. Pharm. Pharmacol.* **1988**, *38*, 272.

0022-2623/88/1831-0300$01.50/0 © 1988 American Chemical Society