Development of Conformationally Constrained Linear Peptides Exhibiting a High Affinity and Pronounced Selectivity for δ Opioid Receptors[1]

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A series of linear conformationally constrained opioid peptides was designed in an attempt to develop highly selective and potent agonists for the δ opioid receptors. These enkephalin analogues corresponding to the general formula Tyr-D-X(OY)-Gly-Phe-Leu-Thr(OZ) were obtained by incorporating bulky residues (X = Ser or Thr; Y = *tert*-butyl or benzyl; Z = *tert*-butyl) into the sequence of the previously reported δ specific agonists DSLET (Tyr-D-Ser-Gly-Phe-Leu-Thr) and DTLET (Tyr-D-Thr-Gly-Phe-Leu-Thr). In binding studies based on displacement of μ and δ opioid receptor selective radiolabeled ligands from rat brain membranes, the two constrained hexapeptides, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr (1, DSTBULET) ($K_I(\mu)$ = 374 nM, $K_I(\delta)$ = 6.14 nM, $K_I(\delta)/K_I(\mu)$ = 0.016) and in particular Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr(*O*-*t*-Bu) (7, BUBU) ($K_I(\mu)$ = 475 nM, $K_I(\delta)$ = 4.68 nM, $K_I(\delta)/K_I(\mu)$ = 0.010) were shown to be among the most potent and selective δ probes reported to date. A roughly similar pattern of selectivity was obtained with the guinea pig ileum and mouse vas deferens bioassays. In addition, the analgesic potency (hot-plate test) of these peptides intracerebroventricularly administered in mice was shown to be significantly related to their μ-receptor affinity.

It has been well established by both pharmacological[2] and receptor–radioligand binding[3] approaches that the endogenous opioid peptides [Met]- and [Leu]enkephalin interact with at least two types of (eventually subdivided) receptors (μ and δ) while dynorphin and its fragments are able to recognize a third binding site designated κ receptor.[4] However, the physiological roles of each receptor type remain to be completely clarified, and to achieve this, highly potent and selective probes are required.[5,6] The rather weak δ selectivity observed with the endogenous opioid effectors can be related to the flexibility of these short linear peptides allowing their easy adaptation to the various receptors types. Therefore, efforts to develop specific ligands with little or no cross reactivity between the various kinds of receptor have been mainly focused on modifications of the enkephalin sequences.[7]

Among the several enkephalin-related peptides, characterized by a wide range of affinity for the different classes of opioid receptors, two types of molecules have been found to display a high selectivity for one kind of site. The first includes linear peptides obtained by modifications of the sequence of the endogenous peptides after extensive conformational studies of the native peptides,[8] leading to proposed requirements for preferential recognition of μ or δ opioid receptors.[9] Among these compounds, the most representative are the μ agonists DAGO,[10] TRIMU 5,[11] and morphiceptin,[12] and the first reported selective δ agonists DSLET[13] and DTLET.[6]

The second type of selective probes belongs to the group of cyclic peptides (review in ref 14 and 15). In the case of the enkephalins, cyclization has essentially led to μ agonists as for example Tyr-D-Orn-Phe-Asp-NH_2.[16] The only exceptions are Tyr-D-Pen-Gly-Phe-Pen (DPLPE) and Tyr-D-Pen-Gly-Phe-D-Pen (DPDPE),[17] which appear even more δ selective than the linear hexapeptides DSLET and DTLET, but have been shown to exhibit a weaker affinity for δ receptors.[18–20] This suggests that the interaction of the conformationally constrained, cyclic penicillamine-containing peptides with the μ receptor is strongly hampered whereas δ recognition is not so highly affected. These assumptions are supported by the results of a comparative conformational NMR study of DTLET and DPLPE,[21] suggesting that the higher δ selectivity of DPLPE as compared to that of DTLET is not due to large differences in the solvated forms of both peptides but can be attributed to highly unfavorable conformational changes required for the cyclic compound to fit the μ binding site.

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(1) Symbols and abbreviations are in accordance with recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature: *Biochem. J.* **1984**, *219*, 245. The following other abbreviations have been used: FAB, fast-atom bombardment; THF, tetrahydrofuran; MeOH, methanol; EtOAc, ethyl acetate; AcOH, acetic acid; Et_2O, ether; DCC, cyclohexylcarbodiimide; HOBT, 1-hydroxybenzotriazole; OHNSu, *N*-hydroxysuccinimide; TFA, trifluoroacetic acid; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Pen, penicillamine; [Met]enkephalin, Tyr-Gly-Gly-Phe-Met; [Leu]enkephalin, Tyr-Gly-Gly-Phe-Leu; DAGO, Tyr-D-Ala-Gly-N(Me)Phe-Gly-Ol.

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Table I. Potency of Opioid Peptides To Inhibit the Binding of [^{3}H]DAGO and [^{3}H]DTLET from Rat Brain Tissue at 35 °C

compound	K_I, nM		K_I([^{3}H]DTLET)/ K_I([^{3}H]DAGO)
	[^{3}H]DAGO	[^{3}H]DTLET	
[Met]enkephalin	27.5 ± 8.0	7.30 ± 2.10	0.27
DSLET	31.0 ± 5.0	4.80 ± 0.80	0.15
DTLET	23.3 ± 2.5	1.35 ± 0.15	0.053
1, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr (DSTBULET)	374 ± 35	6.14 ± 0.73	0.016
2, Tyr-D-Ser(*O*-Bzl)-Gly-Phe-Leu-Thr	42.1 ± 7.7	1.47 ± 0.22	0.035
3, Tyr-D-Thr(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	4500 ± 920	866 ± 120	0.19
4, Tyr-L-Thr(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	>100 000	13 000 ± 380	
5, Tyr-D-Ser-Gly-Phe-Leu-Thr(*O*-*t*-Bu) (DSLETBU)	185 ± 30	6.35 ± 0.90	0.034
6, Tyr-D-Thr-Gly-Phe-Leu-Thr(*O*-*t*-Bu)	64.6 ± 1.8	3.10 ± 0.53	0.048
7, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr(*O*-*t*-Bu) (BUBU)	475 ± 79	4.68 ± 0.60	0.010
8, Tyr-D-Pen-Gly-Phe-Pen	873 ± 210	10.9 ± 1.2	0.012
9, Tyr-D-Pen-Gly-Phe-D-Pen	993 ± 151	19.2 ± 1.4	0.019

Table II. Inhibitory Potencies (IC_{50}) of Opioid Peptides on the Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assays

compound	IC_{50}, nM		IC_{50} MVD/IC_{50} GPI
	GPI	MVD	
[Met]enkephalin	200 ± 19	13 ± 1.5	0.065
DSLET	406 ± 46	0.40 ± 0.04	0.001
DTLET	460 ± 60	0.15 ± 0.012	0.0003
1, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr (DSTBULET)	1795 ± 205	1.07 ± 0.09	0.0006
2, Tyr-D-Ser(*O*-Bzl)-Gly-Phe-Leu-Thr	83.3 ± 11	0.35 ± 0.04	0.0042
3, Tyr-D-Thr(*O*-*t*-bu)-Gly-Phe-Leu-Thr	11 366 ± 300	1194 ± 125	0.10
4, Tyr-L-Thr(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	NT	NT	
5, Tyr-D-Ser-Gly-Phe-Leu-Thr(*O*-*t*-Bu) (DSLETBU)	202 ± 35	0.84 ± 0.10	0.0041
6, Tyr-D-Thr-Gly-Phe-Leu-Thr(*O*-*t*-Bu)	204 ± 43	0.75 ± 0.09	0.0037
7, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr(*O*-*t*-Bu) (BUBU)	2790 ± 400	0.60 ± 0.07	0.000 21
8, Tyr-D-Pen-Gly-Phe-Pen	10 000 ± 900	2.50 ± 0.50	0.000 25
9, Tyr-D-Pen-Gly-Phe-D-Pen	25 250 ± 1089	3.40 ± 0.15	0.000 13

Moreover, a comparison of the conformations of DTLET and DPLPE has shown that the two threonine side chains of DTLET could play the same role as that of the two penicillamino residues.[21,22]

It was therefore decided to increase the steric size of the residues in position 2 or/and 6 of DSLET or DTLET in order to reinforce the structural analogy with the cyclic peptide in an attempt to increase δ selectivity while still retaining the affinity.

In the present study, we describe the synthesis and pharmacological properties of a new family of hexapeptides characterized by introduction of bulky groups on their hydroxylic functions. The pharmacological profile was determined by (i) inhibition of binding in rat brain tissue of [^{3}H]DAGO (specific μ ligand[10]) and [^{3}H]DTLET (δ marker[23]) and (ii) inhibition of the electrically evoked contractions of the guinea pig ileum (GPI) and the mouse vas deferens (MVD) used as relatively selective bioassays for μ and δ receptors, respectively.

Finally, the pharmacological profile of these compounds was assessed by evaluation of their analgesic potency on the hot-plate test after icv administration in mice.

Results and Discussion

At the central level, the activity of the synthesized opioid peptides was evaluated by their ability to inhibit the binding of [^{3}H]DAGO and [^{3}H]DTLET to rat brain membranes (Table I). Their potency on the electrically evoked contractions of the myenteric plexus longitudinal muscle preparation of guinea pig ileum and mouse vas deferens is reported in Table II.

As discussed before, the aim of the present study was to develop new δ-selective linear peptides from DSLET or DTLET sequences by increasing the steric size of their residues in position 2 or/and 6. The choice of the first bulky group used was directed by an unexpected result observed in our previously described series of μ agonists named TRIMU, of general formula: Tyr-D-X-Gly-NH-$CH(CH_3)CH_2CH(CH_3)_2$.[24,25] Within this family of compounds, it was observed that, according to our previously reported model of the μ pharmacophore,[9] the compound TRIMU 4 bearing a D-Ala residue (D-X = D-Ala) is a highly μ selective probe while substitution by an hydrophoilic moiety (D-X = D-Ser) causes a fall in μ selectivity, essentially by decreasing the μ recognition.[11] Following this, the incorporation of a lipophilic *tert*-butyl group on the hydroxylic function of D-Ser^2 was expected to induce a back shift toward μ-receptor interaction. Contrastingly, Tyr-D-Ser(*O*-*t*-Bu)-Gly-NH-$CH(CH_3)CH_2CH(CH_3)_2$ exhibited a weaker μ selectivity than the D-Ser^2 analogue.[11] A comparative NMR study has shown that this change in biological properties was related to a conformational change in the D-Ser(*O*-*t*-Bu)^2 analogue as compared to the D-

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Ser[2]-containing parent compound.[11] Indeed significant differences occurred in the NMR spectra of the two compounds monitored under strictly identical conditions (differences in chemical shifts of αH-Tyr, NH-Gly, βH-Tyr and βH-D-Ser, and in $^3J_{NH-H\alpha}$ of Ser residues). Moreover, the conformational differences were confirmed by the NOE peaks observed in ROESY experiments.

Taking these results into account, Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr (DSTBULET, 1) was synthesized. At both the central and peripheral levels (Tables I and II), the selectivity of 1 was consistent with our initial hypothesis. As shown in Table I, DSTBULET exhibited a similar specificity to that of DPLPE related to its higher potency to displace [^{3}H]DTLET from rat brain tissue than the cyclic peptide. As compared to its parent compound (DSLET), the increase of selectivity (10-fold) of 1 is essentially due to its weaker affinity for μ sites. This strongly suggests that substitution of the hydroxylated residue in position 2 by a bulky group inhibits the μ opioid receptor binding without any significant change in δ affinity, thus providing a highly selective probe ($K_I(\delta)/K_I(\mu) = 0.016$).

A similar shift toward δ selectivity was obtained by replacement of the *tert*-butyl group by a benzyl group in compound 2. Nevertheless, the weaker decrease in δ affinity of 2 than that observed for compound 1 suggests that the steric size of the benzyl group is not sufficient to greatly hinder the conformation involved in μ-receptor interaction or that the benzyl group can adopt a less unfavorable orientation within the μ-binding subsite. In line with this, the *tert*-butyl group was retained as the most appropriate hindering group and was introduced into the DTLET sequence, leading to compound 3, which was expected to be more specific than 1, given the higher selectivity of DTLET as compared to DSLET. Although compound 3 remained relatively δ selective ($K_I(\delta)/K_I(\mu) = 0.18$), it surprisingly lost more than 2 orders of magnitude of affinity for both receptors, suggesting a drastic conformational change in the N-terminal part of the peptide. This assumption was confirmed by a comparative conformational NMR study,[26] showing a large difference between the φ, ψ values for D-Ser[2] (+68°, +22°) and Gly (−95°, −45°) in 1 as compared to those for D-Thr[2] (+40°, +70°) and Gly (−95°, −120°) in 3. Therefore, the loss of affinity of compound 3 is probably due to the close vicinity of the methyl and *tert*-butyl groups, which strongly reduces the accessible conformational space around the D-Thr(*O*-*t*-Bu) residue therefore hindering the interaction with the subsites complementary to the N-terminal Tyr[1] and X[2] amino acids.[26]

Moreover, in compound 4, replacement of D-Thr(*O*-*t*-Bu)[2] by L-Thr(*O*-*t*-Bu) was not able to restore any activity but causes a complete loss of potency (μ or δ), in agreement with the widely observed lack of activity of enkephalin-related peptides possessing a L residue in position 2.[27] As previously discussed, the Thr[6] residue in linear hexapeptides was shown to play the same topological role as Pen[5] in DPLPE.[21,22] Therefore an *O*-*tert*-butyl group was introduced in the sixth amino acid of DSLET and DTLET. In both cases, this modification led to an improved selectivity (compounds 5 and 6). Furthermore, it was noticed that 5 and 6 exhibit a selectivity ratio of the same order (0.034 and 0.048) but differ from one another in affinity. Indeed, replacement of Ser[2] by Thr[2] in 6 provided a marked increase in apparent affinity (K_I) for the two receptors as previously observed for DSLET and DTLET.[6]

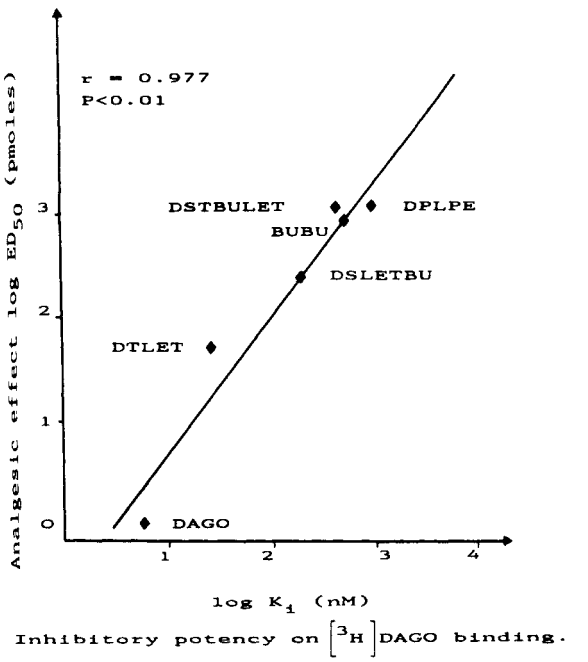

Figure 1. Analgesic effects of opioid peptides: DAGO (ED_{50} = 1 pmol), DTLET (ED_{50} = 50 pmol), DSLETBU (ED_{50} = 261 pmol), BUBU (ED_{50} = 896 pmol), DPLPE (ED_{50} = 1140 pmol), and DSTBULET (1160 pmol). All peptides were injected icv 10 min before exposing animals to the hot plate. Each point is the mean value obtained with 10–20 mice.

Finally, since conformational studies have shown that the amino acids 2 and 6 in linear peptides were mimicking the Pen residues in DPLPE,[21,22] two *tert*-butyl groups were introduced in positions 2 and 6 in DSLET, leading to compound 7, which was found to yield one of the higher δ selectivities so far reported ($K_I(\delta)/K_I(\mu) = 0.010$). This result, mainly due to an increase of the K_I value vs [^{3}H]-DAGO binding, indicates an additive effect of the two bulky groups and suggests that these moieties are able to induce in 7 a conformation similar to that of DPLPE, in inhibiting the μ-receptor recognition. This hypothesis is now confirmed by NMR study in our laboratory.[26] Furthermore, the occurence in 1 and 5–7 of a Gly moiety not included in a bridge, preserves a higher degree of flexibility in the linear peptides. This could explain a less energy consuming recognition of the δ-site for the linear peptide as for the cyclic ones.

Moreover, a comparison of the results in Table II with those in Table I, shows that there is a qualitatively good agreement between the results of in vitro pharmacological assays and the binding data. This is in agreement with the full agonist properties of the described enkephalin-related compounds.

Analgesic Effects. A number of studies[25,28,29] have shown that the analgesic potency (ED_{50} in the hot-plate test) of some selective enkephalin analogues is significantly correlated with their inhibitory effect on the GPI (IC_{50}) and their ability to inhibit [^{3}H]DAGO binding (K_i) in rat brain tissue.

Consequently, each new selective compound (μ or δ) can be tested for its ability to fit a correlation previously es-

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tablished[11] with the actually most selective probes, in order to confirm the preferential involvement of μ receptors in the supraspinal control of pain.

The ED_{50} values of DAGO, DTLET, DSLETBU, DSTBULET, BUBU, and DPLPE are reported in Figure 1. The rank order of potency was found to be DAGO > DTLET > DSLETBU > BUBU > DSTBULET $\simeq$ DPLPE. DAGO is 50 times more potent than DTLET and 1160 times than DSTBULET, and the analgesic effect induced by these peptides is reversed by naloxone.

These results confirm that supraspinal analgesia is preferentially mediated by μ-receptor activation. Indeed previous studies[28] have shown the failure of ICI 154.129 (δ antagonist) to inhibit the analgesic effect of DTLET. Therefore, as previously discussed[25,28] δ agonists should elicit antinociceptive effects through their residual cross reactivity with μ receptors. This was clearly confirmed by the significant correlation ($r = 0.977$, $p < 0.01$) observed between the analgesic effects of the tested peptides and their inhibitory potencies on [^{3}H]DAGO binding in rat brain (Figure 1). Moreover, it should be noted that it was not possible to determine an ED_{50} for compound **3**, because the high doses used, related to its weak μ affinity, elicited comportemental seizures (catalepsy, etc.) before analgesic effect was obtained.

Conclusion

The introduction of bulky residue(s) in linear enkephalin-related hexapeptides represents a new approach in the design of δ-selective probes, displaying the appropriate criteria to investigate the biochemical and pharmacological properties of the assumed physiological binding site (δ) of endogenous enkephalins. Indeed, their selectivities and high affinities are associated with a satisfactory resistance to peptidases.[30] Moreover, the linear structures of these peptides make them easy to both synthesize and obtain in a tritiated form as shown with [^{3}H]DSTBULET.[30] Finally, these results and the preliminary conformational data obtained with DSTBULET and especially BUBU prompted us to extend this approach to various cyclic sulfhydryl bridge containing peptides belonging to other neuropeptides families.[31,32]

Experimental Section

Chemistry. [Met]enkephalin, Tyr-D-Pen-Gly-Phe-Pen and Tyr-D-Pen-Gly-Phe-D-Pen were from Bachem AG (Switzerland). Their analytical data sheet show that their purity is assessed by TLC (two systems) and by HPLC (purity >99%). The peptide content is quantitated by quantitative amino acid analysis (>95%). The peptides were prepared from protected amino acids (Bachem AG) by the liquid-phase method with *tert*-butyloxycarbonyl, benzyloxycarbonyl, and methyl and benzyl esters as protecting groups and dicyclohexylcarbodiimide with hydroxybenzotriazole as coupling agents.[33]

The structure and the lack of racemization of the compounds and all of the intermediates were established by ^{1}H NMR spectroscopy (Bruker WH 270 and 400 MHz). Complete assignment of ^{1}H NMR signals of all the products was performed by classical double resonance experiments already described for the enkephalins.[34] The purity was checked by thin-layer chromatography on (Merck) silica gel plates in the following solvent systems (v/v): A, chloroform–methanol (9:1); B, chloroform–methanol (15:1); C, BuOH–AcOH–H_2O (4:1:1); D, EtOAc–pyridine–AcOH–H_2O (260:20:6:11); and E, chloroform–methanol (7:3). The products were also tested by HPLC (Waters apparatus) on a reverse-phase μ Bondapak C_{18} column with Et_3N–H_3PO_4 buffer (TEAP, 0.025 M, pH 6.5)/CH_3CN as eluents (flow rate, 1.5 mL/min). The eluated peaks were monitored at 210 nm. Melting point of the crystallized products are reported uncorrected.

Amino acid analysis was carried out on an LKB biochrom 4400 analyzer after hydrolysis by 6 N HCl at 110 °C for 24 h. Mass spectra were recorded on a double-focusing VG 70-250 instrument. The FAB ionization was obtained with a FAB saddle field source (Ion Tech Ltd, Teddington, UK) operated with Xenon at 8 kV and 1 mA. Glycerol or cesium iodide was used for calibration. Accelerating voltage was set at 6 kV, and resolution was 1200. Mass spectra were obtained in different matrices and processed by means of the VG-250 software package.

[^{3}H]DAGO (2.2 Tbq/mmol) and [^{3}H]DTLET (2.1 Tbq/mmol) were from CEA, Gif-sur-Yvette, France, Levorphanol (tartrate) was a generous gift from Hoffmann-La Roche.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-serine.** To a solution of N^{α}-Z-Tyr (6.30 g, 20 mM) in THF (60 mL) cooled in an ice–water bath were added successively a solution of (*O*-*t*-Bu)-D-Ser-OCH_3, HCl (4.24 g, 20 mM), and triethylamine (2.8 mL) in $CHCl_3$ (60 mL), a solution of HOBT (3.06 g, 20 mM) in THF (20 mL), and a solution of DCC (4.12 g, 20 mM) in $CHCl_3$ (20 mL). After 1 h, the mixture was allowed to come to room temperature and stirred overnight. After removal of dicyclohexylurea and evaporation of solvents in vacuo, the residue was dissolved in EtOAc (80 mL) and washed successively with a saturated solution of NaCl (40 mL), a 10% solution of citric acid (4 × 40 mL), water (40 mL), a 10% solution of $NaHCO_3$ (3 × 40 mL), and, finally, with a saturated solution of NaCl (40 mL). The solvent was dried (Na_2SO_4) and evaporated in vacuo. This procedure is designated as the standard treatment. The protected dipeptide was obtained as an oily residue: yield 8.5 g (90%); mp 80–82 °C; TLC R_f (A) 0.65, R_f (B) 0.30.

To the preceding compound (8.30 g, 17.5 mM) in MeOH (30 mL) cooled at 0 °C was added 18 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 6 h. The solution was concentrated in vacuo, diluted with 30 mL of water, filtered, and acidified to pH 2 with 1 N HCl. After extraction of the aqueous solution by EtOAc, the organic layer was dried and evaporated in vacuo. This treatment is designated as "the standard procedure for alkaline hydrolysis". The white solid was recrystallized from EtOAc, yielding 7.75 g (97%) of the pure protected dipeptide: mp 198–130 °C; TLC R_f 0.95; FAB-MS (MH^+) calcd 459, found 459.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-serylglycine.** To a solution of the preceding compound (7.65 g, 16 mM) in THF (40 mL) cooled at 0 °C were added successively a solution of Gly-OMe, HCl (2.0 g, 16 mM), and triethylamine (2.24 mL) in $CHCl_3$ (40 mL), a solution of HOBT (2.45 g, 16 mM) in THF (20 mL), and a solution of DCC (3.3 g, 16 mM) in $CHCl_3$ (20 mL). After 1 h at 0 °C, the mixture was allowed to come to room temperature and then stirred overnight. The reaction was worked up via the standard treatment and yielded a white solid, recrystallized from EtOAc, yielding 5.72 g (67%): mp 88–90 °C; TLC R_f (A) 0.75.

To the preceding compound (3.26 g, 6.15 mM) in MeOH (20 mL) cooled at 0 °C was added 12.3 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 5 h. The reaction was treated via the standard procedure for alkaline hydrolysis and yielded the N-protected tripeptide: 2.3 g (72%); mp 145–146 °C; TLC R_f (C) 0.90; FAB-MS (MH^+) calcd 516, found 516.

***N*-(*tert*-Butyloxycarbonyl)-L-phenylalanyl-L-leucyl-L-threonine Benzyl Ester.** To a solution of N^{α}-Boc-Phe-Leu[35] (3.78 g, 10 mM) in THF (20 mL) were added successively a solution of L-Thr-*O*-Bzl, hemioxalate (2.54 g, 10 mM), and triethylamine (1.4 mL) in $CHCl_3$ (100 mL), a solution of HOBT (1.53

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Table III. Analytical Data of Opioid Peptides Analogues

no.	compound	amino acid anal. Tyr	D-X	Gly	Phe	Leu	Thr	TLC: R_f C	D	HPLC TEAP/CH_3CN, %	elut time	FAB-MS MH^+ calcd	found
1	Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	0.96	1.01	0.98	1.02	1.00	0.96	0.58	0.48	75/25	14.4	743	743
2	Tyr-D-Ser(*O*-Bzl)-Gly-Phe-Leu-Thr	0.95		1.00	0.98	1.00	1.03	0.62	0.50	70/30	122	777	777
3	Tyr-D-Thr(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	0.90	0.85	1.01	1.02	1.00	1.01	0.68	0.55	75/25	12.1	757	757
4	Tyr-L-Thr(*O*-*t*-Bu)-Gly-Phe-Leu-Thr	0.88	0.87	1.03	1.04	1.00	1.01	0.67	0.53	75/25	11.8	757	757
5	Tyr-D-Ser-Gly-Phe-Leu-Thr(*O*-*t*-Bu)	0.98	1.03	1.01	0.85	1.00	0.89	0.75	0.55	75/25	10.8	743	743
6	Tyr-D-Thr-Gly-Phe-Leu-Thr(*O*-*t*-Bu)	0.92	1.00	1.01	0.86	1.00	0.87	0.60	0.48	75/25	11.2	757	757
7	Tyr-D-Ser(*O*-*t*-Bu)-Gly-Phe-Leu-Thr(*O*-*t*-Bu)	0.89	0.86	1.02	1.04	1.00	0.85	0.87	0.60	68/32	9.4	799	799

g, 10 mM), and a solution of DCC (2.06 g, 10 mM). After 0.5 h at 0 °C, the reaction mixture was stirred at room temperature overnight. The reaction mixture was treated as usual and a white solid was obtained: 4.3 g (76%); mp 90–92 °C; TLC R_f (A) 0.69.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-seryl-glycyl-L-phenylalanyl-L-leucyl-L-threonine Benzyl Ester.** The preceding compound (4.20 g, 7.4 mM) was dissolved in TFA (11 mL) at 0 °C. After 30 min, the mixture was allowed to come to room temperature and stirred for 50 min. After evaporation of TFA in vacuo, the addition of ether (100 mL) led to the precipitation of the crude compound. The white solid was washed with ether (5 × 80 mL) and dried in vacuo: yield 4.26 g (99%); TLC R_f (C) 0.83.

To a solution of the preceding compound (2.92 g, 5 mM) and triethylamine in (0.7 mL) $CHCl_3$ (30 mL) were added successively a solution of N^{α}-Z-Tyr-(*O*-*t*-Bu)-D-Ser-Gly (2.57 g, 5 mM) in THF (30 mL), a solution of HOBT (0.77 g, 5 mM) in THF (20 mL), and a solution of DCC (1.03 g, 5 mM) in $CHCl_3$ (20 mL). After 1 h at 0 °C, the reaction mixture was stirred overnight at room temperature. The usual treatment led to the crude diprotected hexapeptide, which was purified by chromatography on Kieselgel 60 with $CHCl_3$–CH_2OH (15/1) as eluent. Fractions containing pure compound were collected: yield 4.35 g (90%); mp 145–146 °C; TLC R_f (A) 0.42, R_f (B) 0.16; FAB-MS (MH^+) calcd 967, found 967.

L-Tyrosyl-*O*-*tert*-butyl-D-serylglycyl-L-phenylalanyl-L-leucyl-L-threonine (1). A sample of the preceding compound (100 mg, 0.10 mM) in 6 mL of MeOH was hydrogenated at room temperature and atmospheric pressure over Pd–C (10%, 20 mg) during 3 h. After removal of the catalyst, the solution was evaporated to dryness, yielding 70 mg of the crude hexapeptide, which was purified by chromatography on Kieselgel 60 with CH_2Cl_2–MeOH–AcOH–H_2O, 7/3/0.6/0.3, as eluent. Fractions containing pure hexapeptide were collected: yield 63 mg (85%). The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

***N*-(*tert*-Butyloxycarbonyl)-*O*-benzyl-D-serylglycyl-L-phenylalanyl-L-leucyl-L-threonine Benzyl Ester.** To a solution of N^{α}-Boc-(*O*-Bzl)-D-Ser (0.23 g, 0.78 mM) in THF (10 mL) were added successively a solution of Gly-Phe-Leu-Thr-*O*-Bzl, TFA[35] (0.5 g, 0.78 mM), and triethylamine (0.11 mL) in $CHCl_3$ (10 mL), a solution of HOBT (0.12 g, 0.78 mM) in THF (5 mL), and a solution of DCC (0.16 g, 0.78 mM) in $CHCl_3$ (5 mL). After 1 h at 0 °C, the reaction mixture was stirred overnight and then treated as usual. A white solid was obtained: 0.48 g (76%); mp 140–142 °C; TLC R_f (A) 0.50, R_f (B) 0.23.

***N*-(*tert*-Butyloxycarbonyl)-L-tyrosyl-*O*-benzyl-D-seryl-glycyl-L-phenylalanyl-L-leucyl-L-threonine Benzyl Ester.** As usual, the *N*-*tert*-butyloxycarbonyl group of 0.47 g of the preceding compound was removed by TFA, yielding 0.41 g (87%) of the trifluoroacetate salt. To this compound (0.31 g, 0.38 mM) and triethylamine (54 μL) in $CHCl_3$ (10 mL) were added successively, at 0 °C, a solution of N^{α}-Boc-Tyr (0.11 g, 0.38 mM) in THF (10 mL), a solution of HOBT (0.06 g, 0.38 mM) in THF (5 mL), and a solution of DCC (0.08 g, 0.38 mM) in $CHCl_3$ (5 mL). After 1 h at 0 °C, the reaction mixture was stirred overnight and then treated as usual. The crude product was purified by "flash chromatography" on Kieselgel 60 with $CHCl_3$–MeOH, 19/1, as eluent. Fractions containing pure diprotected hexapeptide were collected: yield 0.26 g (70%); mp 158–160 °C; TLC R_f (A) 0.36, R_f (B) 0.15; FAB-MS (MH^+) calcd 967, found 967.

L-Tyrosyl-*O*-benzyl-D-serylglycyl-L-phenylalanyl-L-leucyl-L-threonine (2). To a sample of the preceding compound (0.12 g, 13 mM) in MeOH (5 mL), cooled at 0 °C, was added 0.26 mL of 1 N NaOH. The mixture was stirred at room temperature for 6 h and treated via the standard procedure for alkaline hydrolysis to yield 90 mg (80%) of a white solid: TLC R_f (B) 0.85, R_f (D) 0.65. A sample of this compound (88 mg, 0.1 mM) was deprotected by dissolution in TFA (0.2 mL) at 0 °C. After 30 min, the mixture was allowed to come to room temperature and stirred for 30 min. The addition of ether (50 mL) led to the precipitation of the crude compound. The white solid was washed with ether (2 × 30 mL) and dried in vacuo: yield 80 mg (91%). The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-threonine.** To a solution of N^{α}-Z-Tyr (1.58 g, 5 mM) in THF (25 mL) cooled at 0 °C were added successively a solution of D-Thr-(*O*-*t*-Bu)-OMe, HCl (1.13 g, 5 mM), and triethylamine (0.70 mL) in $CHCl_3$ (25 mL), a solution of HOBT (0.76, 5 mM) in THF (10 mL), and a solution of DCC (1.03 g, 5 mM) in $CHCl_3$ (10 mL). After 1 h, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the standard treatment and yielded 2.23 g (92%) of the pure compound: mp 96–98 °C; TLC R_f (A) 0.62, R_f (B) 0.30.

To the preceding compound (2.2 g, 4.5 mM) in MeOH (9 mL) cooled at 0 °C was added 9.1 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 7 h. Then, the reaction mixture was treated via the standard procedure for alkaline hydrolysis: yield 1.85 g (86%); mp 132–133 °C; TLC R_f (C) 0.97, R_f (D) 0.75; FAB-MS (MH^+) calcd 473, found 473.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-L-threonine Benzyl Ester.** To the preceding compound (0.24 g, 0.5 mM) in THF (25 mL) were added successively a solution of Gly-Phe-Leu-Thr-*O*-Bzl, TFA[35] (0.32 g, 0.5 mM), and triethylamine (0.07 mL) in $CHCl_3$ (25 mL), a solution of HOBT (0.08 g, 0.5 mM) in THF (10 mL), and a solution of DCC (0.11 g, 0.5 mM) in $CHCl_3$ (10 mL). After 1 h at 0 °C, the reaction was stirred overnight at room temperature. The usual treatment led to the crude diprotected hexapeptide, which was purified by "flash chromatography" on Kieselgel 60 with $CHCl_3$–MeOH, 20/1, as eluent. Fractions containing pure compound were collected: yield 0.40 g (82%); mp 162–164 °C; TLC R_f (A) 0.53, R_f (B) 0.25; FAB-MS (MH^+) calcd 981, found 981.

L-Tyrosyl-*O*-*tert*-butyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-L-threonine (3). A sample of the preceding compound (0.06 g, 0.06 mM) was deprotected as described for 1, yielding 35 mg (76%) of the pure hexapeptide. The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

L-Tyrosyl-*O*-*tert*-butyl-L-threonylglycyl-L-phenylalanyl-L-leucyl-L-threonine (4). This compound was synthesized by a similar way as its diastereoisomeric parent **3**. The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-D-serine Methyl Ester.** To a solution of N^{α}-Z-Tyr (8.78 g, 25 mM) in THF (100 mL) cooled at 0 °C were added successively a solution of D-Ser-OMe, HCl (3.90 g, 25 mM), and triethylamine (3.5 mL) in CH_2Cl_2 (100 mL), a solution of HOBT (3.82 g, 25 mM) in THF (10 mL), and a solution of DCC (5.15 g, 25 mM) in CH_2Cl_2 (10 mL). After 1 h at 0 °C, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the

standard treatment and yielded 7.5 g (72%) of the pure compound: mp 125–126 °C; TLC R_f (A) 0.45, R_f (B) 0.18.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-D-serylglycine.** To the preceding compound (7.46 g, 18 mM) in MeOH cooled at 0 °C was added 36 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. Usual treatment of the reaction mixture led to 6.41 g of the attempted compound. To this compound (6.37 g, 15.8 mM) in THF (30 mL), cooled at 0 °C, were added successively a solution of Gly-OCH_3, HCl (1.41 g, 15.8 mM), and triethylamine (2.22 mL) in CH_2Cl_2 (30 mL), a solution of HOBT (2.42 g, 15.8 mM) in THF (20 mL), and a solution of DCC (3.26 g, 15.8 mM) in CH_2Cl_2 (20 mL). After 1 h at 0 °C, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the standard treatment and yielded 5.8 g (77%) of a white solid: mp 95–96 °C; TLC R_f (A) 0.33, R_f (D) 0.74.

To a sample of the preceding compound (1.18 g, 2.56 mM) in MeOH, cooled at 0 °C, was added 5 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 3 h. Usual treatment led to 0.82 g (70%) of the N-protected tripeptide: mp 140–141 °C; TLC R_f (C) 0.90, R_f (B) 0.50; FAB-MS (MH^+) calcd 460, found 460.

***N*-(Benzyloxycarbonyl)-L-phenylalanyl-L-leucine Methyl Ester.** To a solution of N^{α}-Z-Phe (7.50 g, 25 mM) in THF (100 mL) cooled at 0 °C were added successively a solution of L-Leu-OCH_3, HCl (4.55 g, 25 mM), and triethylamine (3.5 mL) in CH_2Cl_2 (100 mL), a solution of HOBT (3.82 g, 25 mM), and a solution of DCC (5.15 g, 25 mM) in CH_2Cl_2 (10 mL). After 1 h at 0 °C, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the standard treatment and yielded 9.6 g (90%) of the pure compound: mp 106–108 °C; TLC R_f (A) 0.75, R_f (B) 0.38.

***N*-(Benzyloxycarbonyl)-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine Methyl Ester.** To the preceding compound (4.60 g, 10.8 mM) in MeOH cooled at 0 °C was added 11 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 6 h. Usual treatment of the reaction mixture led to 3.8 g of N^{α}-Z-Phe-Leu. To a solution of this compound (2.0 g, 4.85 mM) in THF (20 mL) cooled at 0 °C were added successively a solution of (*O*-*t*-Bu)-L-Thr-OCH_3, HCl (1.1 g, 4.85 mM), and triethylamine (0.68 mL) in CH_2Cl_2 (20 mL) and a solution of DCC (1.0 g, 4.85 mM) in CH_2Cl_2 (5 mM). After 1 h at 0 °C, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the standard treatment and yielded 2.4 g (84%) of the pure compound: mp 90–92 °C; TLC R_f (A) 0.64, R_f (B) 0.30.

L-Phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine. To a solution of the preceding compound (2.20 g, 3.77 mM) in MeOH (10 mL) cooled at 0 °C was added 7.6 mL of 1 N NaOH. The mixture was stirred at 0 °C for 1 h and at room temperature for 16 h. Usual treatment of the reaction mixture led to 1.86 g of the N-protected tripeptide. This compound was deprotected by hydrogenolysis as described for 1, yielding 1.35 g (95%): mp 120–125 °C; TLC R_f (C) 0.65, R_f (D) 0.32. Amino acid anal.: Phe, 1.02 (1.0); Leu, 1.0 (1.0); Thr, 0.87 (1.0).

***N*-(Benzyloxycarbonyl)-L-tyrosyl-D-serylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine.** To a solution of N^{α}-Z-Tyr-D-Ser-Gly (0.46 g, 1 mM) in DMF (5 mL) cooled at −20 °C, was added successively OHNSu (0.12 g, 1 mM) and DCC (0.23 g, 1.1 mM). After 0.5 h at −20 °C and 1 h at 0 °C, the reaction mixture was allowed to come to room temperature and stirred overnight. Then DCU was removed by filtration and Phe-Leu-(*O*-*t*-Bu)-Thr (0.45 g, 1 mM) was added at 0 °C. After 1 h at 0 °C, the reaction mixture was allowed to come to room temperature and stirred overnight. After filtration and evaporation of DMF in vacuo, the residue was dissolved in AcOEt (20 mL) and washed successively with a 10% solution of citric acid (2 × 20 mL) and a saturated solution of NaCl (25 mL). The solvent was dried on Na_2SO_4 and evaporated in vacuo, leading to the crude compound, which was purified by chromatography on Kieselgel with AcOEt–pyridine–AcOH–H_2O, 260/20/6/11, as eluent. Fractions containing pure compound were collected: yield 0.44 g (50%); mp 165–167 °C; TLC R_f (A) 0.97, R_f (B) 0.46; FAB-MS (MH^+) calcd 877, found 877.

L-Tyrosyl-D-serylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine (5). A sample of the preceding compound (50 mg, 0.06 mM) was deprotected as described for 1, leading to 41 mg (92%) of the pure hexapeptide. The amino cid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-D-threonylglycine Methyl Ester.** To a solution of N^{α}-Z-Tyr-D-Thr[11] (5.30 g, 12.7 mM) in THF (30 mL) were added successively a solution of Gly-OCH_3, HCl (1.13 g, 12.7 mM), and triethylamine (1.78 mL) in $CHCl_3$ (30 mL), a solution of HOBT (1.95 g, 12.7 mM) in THF (20 mL), and DCC (2.62 g, 12.7 mM) in $CHCl_3$ (20 mL). After 1 h at 0 °C, the mixture was allowed to come to room temperature and stirred overnight. The reaction was worked up via the standard treatment, leading to the crude compound, which was purified by "flash chromatography" on Kieselgel 60 with Et-OAc–$CHCl_3$–MeOH, 95/5/3, as eluent, yielding 4.85 g (78%) of the pure diprotected dipeptide: mp 96–98 °C; R_f (A) 0.83, R_f (B) 0.25.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine.** To the preceding compound (2.20 g, 3.77 mM) in MeOH (10 mL) cooled at 0 °C was added 7.5 mL of 1 N NaOH. The mixture was stirred 1 h at 0 °C and at room temperature overnight. The standard procedure for alkaline hydrolysis yielded 1.86 g (87%e of the attempted compound. To a sample of this latter compound (0.47 g, 1 mM) dissolved in DMF (5 mL) and cooled at −20 °C were added OHNSu (0.12 g, 1 mM) and DCC (0.23 g, 1.1 mM). After the mixture was stirred for 0.5 h at −20 °C, 1 h at 0 °C, and 15 h at room temperature and DCU was removed, Phe-Leu-(*O*-*t*-Bu)-Thr (0.45 g, 1 mM) was added. The reaction mixture was stirred 1 h at 0 °C and at room temperature overnight. The treatment and the purification previously described for the precursor of compound 5 led to 0.45 g (50%) of the pure compound: mp 146–148 °C; R_f (A) 0.98, R_f (B) 0.53; FAB-MS (MH^+) calcd 891, found 891.

L-Tyrosyl-D-threonylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine (6). A sample of the preceding compound (50 mg, 0.06 mM) was deprotected as described for 1, leading to 40 mg (95%) of compound 6. The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

***N*-(Benzyloxycarbonyl)-L-tyrosyl-*O*-*tert*-butyl-D-serylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine.** To a solution of N^{α}-Z-Tyr-(*O*-*t*-Bu)-D-Ser-Gly (0.51 g, 1 mM) in DMF (5 mL) cooled at −20 °C were added OHNSu (0.12 g, 1 mM) and DCC (0.23 g, 1.1 mM). After the mixture was stirred for 0.5 h at −20 °C, 1 h at 0 °C, and at room temperature overnight and DCU was removed, Phe-Leu-(*O*-*t*-Bu)-Thr (0.45 g, 1 mM) was added. The reaction mixture was then stirred for 1 h at 0 °C, and the purification previously described for the precursor of compound 5 led to 0.56 g (60%) of the pure compound: mp 214–216 °C; R_f (D) 0.80, R_f (B) 0.72; FAB-MS (MH^+) calcd 933, found 933.

L-Tyrosyl-*O*-*tert*-butyl-D-serylglycyl-L-phenylalanyl-L-leucyl-*O*-*tert*-butyl-L-threonine (7). A sample of the preceding compound (50 mg, 0.05 mM) in 4 mL of MeOH was hydrogenated at room temperature and atmospheric pressure over PdO (5 mg) during 3 h. After removal of the catalyst, the solution was evaporated to dryness, yielding 40 mg (95%) of pure compound 7. The amino acid analysis, TLC, analytical HPLC, and FAB-MS data are given in Table III.

Preparation of Membrane Fraction and Binding Assays. Membrane fraction was prepared as previously described.[11] Binding studies were carried out in 50 mM Tris·HCl (pH 7.4). Each assay contained 0.6–0.7 mg of protein, the radioligand ([^{3}H]DAGO, 1 nM; [^{3}H]DSLET, 2 nM), and other additions in a final volume of 1 mL. All points were determined in triplicate, and the nonspecific binding was measured in the presence of 10 μM levorphanol. After a 45-min incubation at 35 °C, the contents of each tube were rapidly filtered over Whatman GF/B filters. The filters were washed twice with 5 mL of cold Tris·HCl buffer, dried, and suspended in 5 mL of scintillation liquid (Beckman Ready Solv EP Cocktail), and bound radioactivity was counted by a 1209 Rackbeta (LKB) (counting efficiency ≃ 55%).

Data Analysis. The specific binding of tritiated ligands was defined as the difference in the means of triplicate determinations of the binding, measured in the presence and the absence of 10 μM levorphanol. Competition curves were fitted by linear regression analysis of the Hill transformation. K_i values were

calculated according to the Cheng–Prusoff relationship,[36] assuming competitive interactions.

Biological Tests. The compound were assayed for their μ and δ opioid activities on the GPI and MVD respectively as previously described.[37] The agonist concentration that produces half-maximal inhibition of the electrically stimulated muscle twitch, IC_{50}, was determined from six to eight computed log dose–response curves with six different concentrations of the compound. Since the sensitivity varied from one tissue to another, [Met]enkephalin IC_{50} was determined on the same preparation, and the agonist IC_{50} was corrected by multiplication of the ratio: mean of all the [Met]enkephalin IC_{50}/[Met]enkephalin IC_{50} of the assay.

Analgesic Tests. Analgesic potencies were evaluated on the hot-plate test at 55 ± 0.2 °C by using albino mice (Deprē, France) weighing 24–28 g.[28] The hot plate was surrounded by a cylindrical plexiglass chimney (14-cm diameter; 20-cm high). The jump latency time was measured by means of a stopwatch, 10 min after intracerebroventricular (icv) drug administration by hand (10-μL volume) with a modified 30-gauge needle and a Hamilton syringe.[38] Mice that did not jump within 180 s were removed (cut-off time). The experimenter was blind with respect to pretest manipulations. The percentage of analgesia was calculated according to the formula $(T_t - T_c)/(180 - T_c)$ (T_t and T_c are the jump latency times of treated and control animals respectively). The cut-off time (180 s) represents 100% analgesia. The ED_{50} and their 95% confidence limits were determined according to the method of Litchfield and Wilcoxon.[39] Each value represented 10–20 mice tested. Correlation between the analgesic effect of opioid peptides and their ability to inhibit [^{3}H]DAGO binding on crude brain membrane preparation was assessed by a multiple linear regression analysis.

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Synthesis and Biological Evaluation of Isomeric Dinucleoside Monophosphates and Monomethylphosphonates of 9-β-D-Arabinofuranosyladenine and Related Analogues[1]

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The 3′→5′, 3′→3′ and 5′→5′ dinucleoside monophosphates and methylphosphonates of 9-β-D-arabinofuranosyladenine, as well as its 5′-(hydrogen phosphonate) and 5′-(methyl methylphosphonate) derivatives have been the subject of a systematic synthesis and examination of their biological, i.e. antiviral and cytostatic, properties. First the properly protected monomeric building blocks were prepared and then condensed to give fully protected intermediates. These latter were then deblocked to afford the unprotected compounds, which were fully characterized. Only the 3′→5′ phosphodiester isomers **13** and **16** and, to a lesser extent, the 5′-(hydrogen phosphonate) derivative **21** showed marked biological activity.

Of the various nucleoside analogues that have been described as antiviral and antitumor agents,[2] 9-β-D-arabinofuranosyladenine (araA, vidarabine, VIRA-A) is one of the best known drugs with rather selective antiherpetic activity.[3] Although araA has been licensed for clinical use, i.e. for the topical treatment of herpetic keratitis and the systemic intravenous treatment of herpetic encephalitis,

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