Analgesic Dipeptides VI.: Synthesis and Structure-Activity Relationships of N-Terminal Modified Analogues of the Analgesic Compounds H-Xaa-Trp(Nps)-OMe (Xaa=Lys, Orn, Arg)**)

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In order to determine the influence of the N-terminal amino group of the dipeptide derivatives H-Xaa-Trp(Nps)-OMe[Xaa=Lys (**2a**), Orn (**2b**), Arg (**2c**)] on their antinociceptive effects, the syntheses of their corresponding deaminated, acetylated and dimethylated analogues have been achieved. Deamino and dimethyl analogues of **2a,b**, **6a,b**, and **18a,b** were prepared by coupling the corresponding N^{ω}-Z- and N^{ω}-Z-N^{α}-Me_2 amino acids with H-Trp-OMe, using the DCC/HOSu method, followed by sulfenylation of the resulting compounds and removal of the Z groups. Guanidylation of **6b** and **18b** provided the arginine analogues **6c** and **18c**, respectively. Ac-Xaa-Trp(Nps)-OMe [Xaa=Lys (**11a**), Orn (**11b**) were synthesized by acetylation of H-Xaa(Z)-Trp(Nps)-OMe with acetic anhydride, in the presence of 4-dimethylaminopyridine, and subsequent removal of the Z groups. Coupling of Ac-Arg-OH·HCl with H-Trp-OMe, using the DCC/HOSu procedure, followed by sulfenylation of the resulting 8:3 diastereomeric mixture of L,L and L,D dipeptides afforded Ac-*ambo*-Arg-Trp(Nps)-OMe **11c+11d**. The antinociceptive effects of **6a-c**, **11a-d**, **and 18 a-c** were evaluated after i.c.v. administration in mice. The N^{α}-acetyl dipeptides **11** were found to exhibit a naloxone-reversible antinociceptive effects comparable with those of **2**, while N-deaminated and N, N - dimethylated analogues were inactive.

Dipeptide als Analgetika, 6.Mitt.: Synthese und SAR-Studien an N-terminal modifizierten Analoga des Analgeticums H-Xaa-Trp(Nps)-OMe (Xaa=Lys, Orn, Arg).

Mit der Absicht, den Einfluß der Endaminogruppe der Dipeptidverbindungen H-Xaa-Trp(Nps)-OMe [Xaa=Lys (**2a**), Orn (**2b**), Arg (**2c**)] auf ihre antinociceptive Wirkung zu prüfen, wurden die entspr. deaminierten, acetylierten und dimethylierten Analoga synthetisiert. Die Deamino- und Dimethylverbindungen von **2a,b**, **6a,b** und **18a,b** wurden über die Kupplung der entspr. N^{ω}-Z- und N^{ω}-Z-N^{α}-Me_2 aminosäuren mit H-Trp-OMe unter Anwendung der DCC/HOSu Methode mit anschließender Sulfenylierung der erhaltenen Verbindungen und letzlich Abspaltung der Z-Gruppen synthetisiert. Guanidylierung von **6a** und **18b** führte zu den Argininanaloga **6c** bzw. **18c**. Ac-Xaa-Trp(Nps)-OMe [Xaa=Lys (**11a**), Orn (**11b**)] wurden durch Acetylierung von H-Xaa(Z)-Trp(Nps)-OMe mit Essigsäureanhydrid/4-Dimethylaminopyridin und anschließende Abspaltung der Z-Gruppen erhalten. Die Kupplung von Ac-Arg-OH·HCl mit H-Trp-OMe (DCC/HOSu Methode) und anschließende Sulfenylierung der erhaltenen diastereomeren Mischung (8:3) der L,L bzw. L,D Dipeptide erbrachte Ac-*ambo*-Arg-Trp(Nps)-OMe **11c+11d**. Die antinociceptive Wirkung von **6a-c**, **11a-d** und **18a-c** wurde nach i.c.v. Verabreichung an Mäusen getestet. Die N-Acetyldipeptide **11** zeigten eine Naloxon-reversible antinociceptive Wirkung, vergleichbar der von **2**, während N-deaminierte und N,N-dimethylierte Verbindungen wirkungslos waren.

In the first paper of this series [1], it was reported that i.c.v. administration of the synthetic dipeptide derivatives H-Xaa-Trp(Nps)-OH [Trp(Nps)=2-(*o*-nitrophenylsulfenyl)tryptophan; Xaa=Lys (**1a**), Orn (**1b**), Arg (**1c**)], and their corresponding methyl esters **2a**, **2b** and **2c**, exhibited a naloxone-reversible antinociceptive effect comparable with that of the enkephaline analogue D-Ala^2-Met-enkephalinamide (DAME). Studies on the mechanism of action appear to indicate that these dipeptide derivatives do not act directly on opioid receptors, but their antinociceptive effects could be probably explained by a mixture of peptidase-inhibiting and Met-enkephalin-releasing properties[1]. Several similarities have been recently found between this series of Trp(Nps)-containing dipeptides and the endogeneous opioid dipeptide H-Tyr-Arg-OH (kyotorphin), a Met-enkephalin-releaser[3]. However, the antinociceptive potency of **1** and **2**, when administered i.c.v. in mice, is aproximately 50- fold higher than that of kyotorphin[2]. Preliminary studies to establish the structural requirements for the antinociceptive effect of **1** and **2** showed, besides the importance of the Nps moiety[1,4],

For symbols and abbreviations see lit. [5].

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Table 1. Analytical data of protected dipeptide analogues **4a,b, 5a,b, 8a, 9a, 10a,b, 12c+12d, 16a,b** and **17a,b**

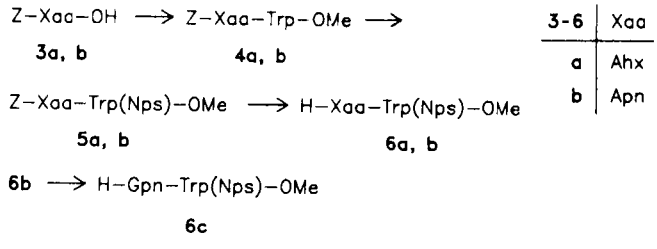
Comp.	Yield %	Mp (°C)	Cald. Found	C	H	N	S	Cl
4a	89	syrup	$C_{26}H_{31}N_3O_5$	67.1	6.71	9.0	-	-
			(465.5)	67.3	6.94	8.7	-	-
4b	71	58-60[a]	$C_{25}H_{39}N_3O_5$	66.5	6.47	9.3	-	-
			(461.6)	66.4	6.65	9.4	-	-
5a	86	62-64[a]	$C_{32}H_{34}N_4O_7S$	62.1	5.54	9.1	5.2	-
			(618.7)	61.9	5.78	8.9	5.1	-
5b	72	58-60[a]	$C_{31}H_{32}N_4O_7S$	61.6	5.33	9.3	5.3	-
			(604.7)	61.3	5.56	9.1	5.2	-
8a	72	syrup	$C_{32}H_{35}N_5O_7S$	60.6	5.57	11.0	5.1	-
			(633.7)	60.9	5.89	10.8	5.3	-
9a	92	115-117[b]	$C_{32}H_{36}ClN_5O_7S$	57.3	5.41	10.4	4.8	5.3
			(670.2)	57.1	5.68	10.2	4.6	5.4
10a	97	92-94[c]	$C_{34}H_{37}N_5O_7S$	61.9	5.65	10.6	4.9	-
			(659.7)	61.8	5.89	10.4	4.8	-
10b	92	86-88	$C_{33}H_{35}N_5O_8S$	59.9	5.33	10.6	4.8	-
			(661.7)	60.0	5.40	10.7	5.1	-
12c+12d	98	-	$C_{20}H_{29}ClN_6O_4$	53.0	6.45	18.5	-	7.8
			(452.9)	52.8	6.71	18.4	-	7.8
16a	87	syrup	$C_{28}H_{36}N_4O_5$	66.1	7.13	11.0	-	-
			(508.6)	65.8	7.35	10.8	-	-
16b	81	syrup	$C_{27}H_{34}N_4O_5$	65.6	6.93	11.3	-	-
			(494.6)	65.3	7.20	11.1	-	-
17a	82	foam	$C_{34}H_{39}N_5O_7S$	61.7	5.94	10.6	4.8	-
			(661.8)	61.5	5.70	10.3	4.9	-
17b·HCl	91	76-78	$C_{33}H_{38}ClN_5O_7S$	57.9	5.60	10.2	4.7	5.2
			(684.2)	58.2	5.77	10.0	4.5	5.2

[a] Crystallized from $CHCl_3$-hexane. [b] Crystallized from EtOAc-ether. [c] Crystallized from EtOAc-hexane.

the need for a basic amino acid[1]. This necessity was evidenced from the total loss of activity which took place, when the basicity of the ε-NH_2 or the guanidino group in **1a** or **1c** was partially masked with a benzyloxycarbonyl (Z) or a N^7,N^8-(1,2-dihydroxyclohex-1,2-ylen) (DHCH) group[1]. Now, in order to determine the influence of the N-terminal amino group on the analgesic effect, the analogues of **2**, which correspond to deletion, acetylation and dimethylation, respectively, of this group, habe been synthesized.

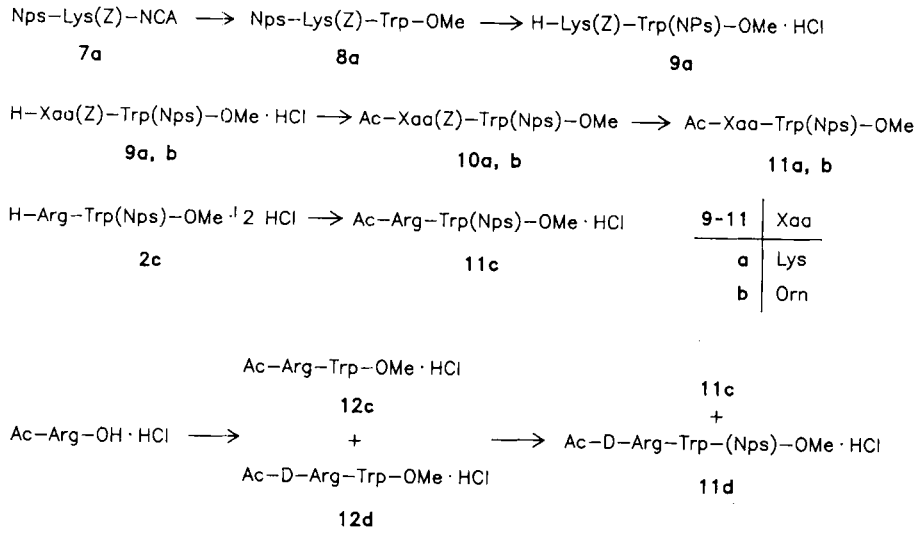
Chemistry

The synthesis of the deamino analogues **6a** and **6b**, which correspond to the deletion of the N-terminal amino group in the lysyl and ornithyl derivatives **2a** and **2b**, was accomplished by the route depicted in Scheme 1, which initally involves the coupling of Z-εAhx and Z- δApn[5] (**3a** and **3b**) to H-Trp-OMe, employing the DCC/HOSu procedure,



to give **4a** and **4b**, respectively. Reaction of **4a** and **4b** with *o*-nitrophenylsulfenyl chloride (Nps-Cl) in N HCl in dioxane provided the Trp(Nps)-containing analogues **5a** and **5b**, which on treatment with boron-tris(trifluoroacetate)/trifluoroacetic acid (BTFA/TFA), as deblocking agent for the cleavage of the Z group[6], afforded the desired compounds **6a** and **6b**, respectively.Guanidylation of the δApn-containing derivative **6b**, using 1-amidino-3,5-dimethylpyrazole nitrate (ADMPN) by a method similar to that reported by *Klausner* et al[7], gave the deamino analogue of the Arg-containing dipeptide **2c**, **6c**.

As shown in Scheme 2, N-terminal acetylated analogues of **2a,b**, **11a,b**, were prepared by acetylation of the $N^ε$-and $N^δ$- protected dipeptides **9a,b** with acetic anhydride in the presence of one equivalent of 4-dimethylaminopyridine, followed by removal of the Z groups of the resulting acetylated derivatives **10a,b**, utilizing BTFA/TFA for **10a** and trimethylsilyl iodide in acetonitrile (Me_3SiI/CH_3CN)[8] for **10b**. The starting H-Lys(Z)-containing dipeptide **9a** was prepared by a procedure similar to that reported for the synthesis of the ornithine analogue **9b**[9],which firstly consisted of the peptide bond formation by the Nps-NCA method[10] to give the Nps-dipeptide derivative **8a**. Removal of the N-protecting Nps group from **8a** and subsequent transfer of this group to the 2-position of the Trp residue was carried out in a one-pot reaction using N HCl/dioxane to afford **9a**.



In order to prepare the N-acetylarginyl derivative **11c**, H-Arg(HCl)-Trp(Nps)-OMe, *in situ* generated by selective liberation of the α-NH_2 group of the corresponding dihydrochloride **2c** with one equivalent of triethylamine, was treated with acetyl chloride in the presence of triethylamine. Although **11c** was really obtained, all attempts to separate this compound from the triethylammonium chloride formed were unsuccessful. Therefore, we devised an alternative route to **11c**, which initially involved the synthesis of the acetylated dipeptide Ac-Arg-Trp-OMe (**12c**) from commercially available Ac-Arg(HCl)-OH. Result of this coupling in DMF, using the DCC/HOSu method was a mixture of **12c** and its D,L diastereomer **12d** in a 8:3 ratio, as estimated by ^{1}H-NMR. Appreciable racemization does not generally occur during this coupling method[11]. However, in this case, the considerable racemization at arginine can be attributed to the following factors: firstly, the tendency of N-acyl amino acids to racemization[12], and secondly, the use of DMF, a solvent which increases the rate of racemization as compared with other solvents, commonly employed in the peptide bond formation, such as THF or dioxane[13], but in which Ac-Arg (HCl)-OH is poorly soluble. Sulfenylation of the diastereomeric mixture **12c,d** with Nps-Cl gave **11c,d**. Separation of the L,L and D,L diastereomers of **11c,d** or **12c,d** was not observed in any of our chromatography experiments. As it will be shown later, in contrast to the Ac-Lys-containing dipeptide **11a**, the ornithine and arginine analogues **11b** and **11c+11d** produced neurotoxic effects at 0.5 μg/mouse. For this reason, it was not considered of interest to attempt new routes to the preparation of pure diastereomer **11c**.

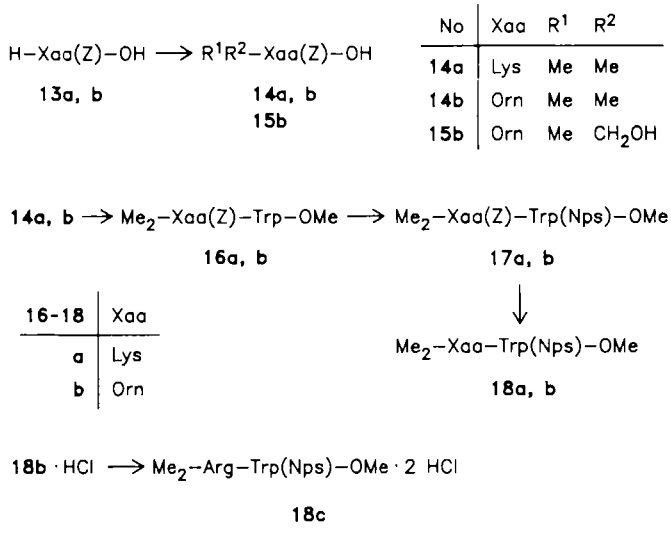
The synthesis of the N,N-dimethyl analogues of **2a,b**, **18a,b**, was accomplished from the corresponding N,N-dimethyl amino acid derivatives **14a,b**, according to Scheme 3. The starting dimethylated lysyl derivative **14a** was prepared following a previously reported method for the reductive dimethylation of primary amines, which consists of the treatment of the amine with aqueous formaldehyde in MeOH and subsequent *in situ* reaction of the resulting imine with $NaBH_4$[14]. In this manner, and using a ratio of the amino acid derivative **13a**: formaldehyde: $NaBH_4$, 1:12.6:3.4, **14a** was obtained in 82% yield. When the ornithine derivative **13b** was used, a higher ratio of formaldehyde (**13b**: formaldehyde, 1:18.6) was required to complete the formation of the imine intermediate. *In situ* reaction of this intermediate with $NaBH_4$, (**13b**: $NaBH_4$, 1:3.4) afforded a mixture of two compounds which, without separation, were identified by ^{1}H-NMR as the expected N,N-dimethyl amino acid **14b** and the N-hydroxymethyl-N-methyl derivative **15b**, in a 1:3 ratio. Thus, among the signals corresponding to the major compound **15b**, appeared two doublets at δ=4.30 and 4.52 (J=14 Hz), each one integrating for one proton, and in the region of the N-CH_3 protons, a singlet at δ2.24, which integrated only for one CH_3 group. As in the case of the dimethylated lysine derivative **14a**, the two CH_3 groups of **14b** appeared as a singlet at δ2.76 ppm. Although N-hydroxyalkyl derivatives are generally unstable, the possibility of hydrogen bond formation between the vicinal hydroxyl and carbonyl groups in **15b** could explain the stability of this compound. A similar explanation has been given in the case of related stable N-hydroxyalkyl α-amino acid derivatives[15]. Several attempts to increase the ratio of **14b** to **15b**, by addition of higher ratios of $NaBH_4$ to the starting amino acid **13b**, once the imine intermediate was formed, were unsuccessful. However, when the isolated mixture of compounds **14b** and **15b** was treated with $NaBH_4$ in a 1:6.8 ratio, compound **15b** was completely transformed into the desired N,N-dimethyl amino acid derivative **14b**.

Finally, coupling of **14a,b** with H-Trp-OMe·HCl, employing the DCC/HOSu method, gave the dipeptide derivatives **16a,b**, which on sulfenylation with Nps-Cl and subsequent cleavage of the Z groups using Me_3SiI/CH_3CN provided the dimethylated dipeptides **18a,b**. In a similar way to that indicated for the preparation of **6c**, guanidylation of **18b** afforded the arginine analogue **18c**.

Table 2. Analytical and UV spectral data of N-terminal deaminated (**6a-c**), acetylated (**11a-d**) and dimethylated (**18a-c**) analogues of H-Xaa-Trp(Nps)-OMe (Xaa=Lys, Orn, Arg)

Comp.	Yield %	Mp (°C)	(EtOH) λ_{max} nm (lgϵ)	Cald. Found	C	H	N	S	Cl
6a	94	foam	356 (3.45)	$C_{24}H_{28}N_4O_5S$	59.5	5.82	11.6	6.6	-
			281 (4.01)	(484.6)	59.2	6.08	11.3	6.4	-
6b	77	foam	356 (3.45)	$C_{23}H_{26}N_4O_5S$	58.7	5.57	11.9	6.8	-
			281 (4.01)	(470.5)	58.4	5.37	11.7	6.7	-
6c	48	syrup	356 (3.44)	$C_{24}H_{28}N_6O_5S$	56.2	5.51	16.4	6.2	-
			281 (4.00)	(512.6)	56.0	5.80	16.2	6.1	-
11a	69	90-92[a]	356 (3.78)	$C_{26}H_{31}N_5O_6S$	57.7	7.77	12.9	5.9	-
			281 (4.01)	(541.6)	57.9	7.79	12.7	5.9	-
11b	88	86[a]	358 (3.53)	$C_{25}H_{29}N_5O_6S$	56.9	5.54	13.3	6.1	-
		(dec.)	281 (4.07)	(527.6)	56.7	5.84	13.0	5.9	-
11c+11d	94	-	355 (3.55)	$C_{26}H_{32}ClN_7O_6S$	51.5	5.32	16.2	5.3	5.8
			280 (4.04)	(606.1)	51.8	5.27	16.4	5.0	5.5
18a	98	88-90[a]	356 (3.40)	$C_{26}H_{33}N_5O_5S$	59.2	6.30	13.3	6.1	-
			281 (4.12)	(527.6)	59.3	6.47	13.2	6.0	-
18b·HCl	94	foam	356 (3.57)	$C_{25}H_{32}ClN_5O_5S$	54.6	5.86	12.7	5.8	6.4
			281 (4.14)	(550.1)	54.3	5.91	12.8	5.5	6.7
18c·2HCl	50	132[b]	356 (3.62)	$C_{26}H_{35}Cl_2N_7O_5S$	49.7	5.61	15.6	5.1	11.3
		(dec.)	281 (4.16)	(628.6)	49.5	5.58	15.3	4.8	11.5

[a] Crystallized from acetone-ether. [b] Crystallized from 2-propanol-ether.



Structural assignments of all the new compounds were made on the basis of their analytical and spectroscopic data. It is interesting to note that the ^{1}H-NMR spectra of all the Trp(Nps)-containing compounds showed a significant shielding of the phenyl H-6 (δ~6.6) of the Nps group (Table 3). This shielding, which is identical with that previously observed in **1a-c**, **2a-c** and in all the related Trp(Nps)-containing dipeptides[2,9], seems to be related to the adoption of a preferential conformation in which the phenyl and the indole rings are not coplanar[2]. The UV-spectra of the desired final compounds **6a-c**, **11a-d** and **18a-c** showed the characteristic absorption maximum of Trp(Nps) derivatives at ~356 nm[16] (Table 2).

Pharmacological Results and Discussion

The antinociceptive effect in mice of all the N-terminal modified analogues of H-Xaa-Trp(Nps) – OMe [Xaa=Lys (**2a**), Orn (**2b**), Arg (**2c**)], here described, given by i.c.v. route has been evaluated. No analgesia was observed with the deamino peptide analogues **6a-c** or with the N-dimethylated derivatives **18a-c**. However, acetylation of the NH_2-terminus of the parent dipeptides **2a-c** also gave analgesic compounds. Table 4 shows the antinociceptive effect of the N-acetylated dipeptides **11a-d**. For comparative purposes, dipeptides **2a-c** have also been included. In all cases, the analgesia was almost completely blocked by previous administration of naloxone, 1mg/kg sc, given 15 min before the i.c.v. injection. Since the new compounds **11a-d** are the N-acetyl derivatives of **2a-c**, the mechanism of action of these derivatives must be quite similar to that found for the deacylated dipeptides[1]. As shown in Table 4, the N-acetyl analogue of the lysine derivative **2a**, **11a**, produced analgesic effects at 0.5-1 µg/mouse similar to those of **2a** at the same doses. These doses of the Ac-Orn and Ac-*ambo*-Arg-containing analogues **11b** and **11c+11d** produced signs of neurotoxicity; therefore, these compounds were studied at 0.25 µg/mouse. With this lower dose, the antinociceptive effects of **11b** and **11c+11d** were quite comparable with those of **11a** and there were no side effects. Dose-related side effects have been previously observed with several compounds of this series of Trp(Nps)-containing dipeptides[1,2]. The fact that N-terminal acetylation of **2a-c** does not significantly affect their antinociceptive effects, while deamination and N,N-dimethylation cause the total loss of activity, seems to indicate that the hydrogen bonding capacity of the N-terminus is a requirement for the analgesic activity of this series of dipeptide derivatives.

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Table 3. ^{1}H-NMR data of dipeptide analogues **4-6, 8-12** and **16-18**

No.	δ(ppm)
4a[a]	2.10 (m, 2H, εAhx α-CH_2); 3.07 (m, 2H, εAhx ε-CH_2); 3.28 (m, 2H, Trp β-CH_2); 3.66 (s, 3H, CO_2CH_3); 4.90 (m, 1H, Trp α-CH); 5.09 (s, 2H, ZCH_2); 7.31 (s, 5H, ZC_6H_5).
4b[a]	2.10 (m, 2H, δApn α-CH_2); 3.08 (m, 2H, δApn δ-CH_2); 3.29 (m, 2H, Trp β-CH_2); 3.66 (s, 3H, CO_2CH_3); 4.86 (m, 1H, Trp α-CH); 5.06 (s, 2H, ZCH_2); 7.30 (s, 5H, ZC_6H_5).
5a[a]	2.00 (m, 2H, εAhx α-CH_2); 3.03 (m, 2H, εAhx ε-CH_2); 3.30 (m, 2H, Trp β-CH_2); 3.63 (s, 3H, CO_2CH_3); 4.83 (m, 1H, Trp α-CH); 5.00 (s, 2H, ZCH_2); 6.63 (dd, 1H, Nps H-6); 7.26 (s, 5H, ZC_6H_5); 8.16 (dd, 1H, Nps H-3).
5b[a]	2.05 (m, 2H, δApn α-CH_2); 3.10 (m, 2H, δApn δ–CH_2); 3.32 (m, 2H, Trp β-CH_2); 3.65 (s, 3H, CO_2CH_3); 4.90 (m, 1H, Trp α-CH); 5.02 (s, 2H, ZCH_2); 6.63 (dd, 1H, Nps H-6); 7.30 (s, 5H, ZC_6H_5); 8.20 (dd, 1H, Nps H-3).
6a[b]	2.00 (m, 2H, εAhx α-CH_2); 2.72 (m, 2H, εAhx ε-CH_2); 3.22 (m, 2H, Trp β-CH_2); 3.49 (s, 3H, CO_2CH_3); 4.50 (dd, 1H, Trp α-CH); 6.69 (dd, 1H, Nps H-6); 8.30 (dd, 1H, Nps H-3).
6b[b]	2.03 (m, 2H, δApn α-CH_2); 2.76 (m, 2H, δApn δ-CH_2); 3.21 (m, 2H, Trp β-CH_2); 3.48 (s, 3H, CO_2CH_3); 4.51 (m, 1H, Trp α-CH); 6.69 (dd, 1H, Nps H-6); 8.29 (dd, 1H, Nps H-3).
6c[b]	2.02 (m, 2H, δGpn α-CH_2); 3.03 (m, 2H, δGpn δ-CH_2); 3.21 (m, 2H, Trp β-CH_2); 3.48 (s, 3H, CO_2CH_3); 4.50 (m, 1H, Trp α-CH); 6.69 (dd, 1H, Nps H-6); 8.29 (dd, 1H, Nps H-3).
8a[c]	3.10 (m, 4H, Lys ε-CH_2 and Trp β-CH_2); 3.60 (s, 3H, CO_2CH_3); 3.40 (m, 1H, Lys α-CH); 5.02 (s, 2H, ZCH_2); 7.30 (s, 5H, ZC_6H_5); 8.30 (dd, 1H, Nps H-3).
9a[c]	2.93 (m, 2H, Lys ε-CH_2); 3.45 (m, 1H, Lys α-CH); 3.47 (s, 3H, CO_2CH_3); 4.55 (m, 1H, Trp α-CH); 4.95 (s, 2H, ZCH_2); 6.68 (dd, 1H, Nps H-6); 7.29 (s, 5H, ZC_6H_5); 8.23 (dd, 1H, Nps H-3).
10a[a]	1.93 (s, 3H, $NHCOCH_3$); 3.67 (s, 3H, CO_2CH_3); 4.33 (m, 1H, Lys α-CH); 4.85 (m, 1H, Trp α-CH); 5.05 (s, 2H, ZCH_2); 6.67 (dd, 1H, Nps H-6); 7.30 (s, 5H, ZC_6H_5); 8.22 (dd, 1H, Nps H-3).
10b[a]	1.90 (s, 3H, $NHCOCH_3$); 3.63 (s, 3H, CO_2CH_3); 4.45 (m, 1H, Orn α-CH); 4.85 (m, 1H, Trp α-CH); 4.96 (s, 2H, ZCH_2); 6.66 (dd, 1H, Nps H-6); 7.30 (s, 5H, ZC_6H_5); 8.19 (dd, 1H, Nps H-3).
11a[b]	1.81 (s, 3H, $NHCOCH_3$); 2.72 (m, 2H, Lys ε-CH_2); 3.22 (m, 2H, Trp β-CH_2); 3.41 (s, 3H, CO_2CH_3); 4.24 (m, 1H, Lys α-CH); 4.48 (dd, 1H, Trp α-CH); 6.68 (dd, 1H, Nps H-6); 8.28 (dd, 1H, Nps H-3).
11b[b]	1.83 (s, 3H, $NHCOCH_3$); 2.74 (m, 2H, Orn δ-CH_2); 3.23 (m, 2H, Trp β-CH_2); 3.34 (s, 3H, CO_2CH_3); 4.31 (m, 1H, Orn α-CH); 4.49 (m, 1H, Trp α-CH); 6.69 (dd, 1H, Nps H-6); 8.29 (dd, 1H, Nps H-3).
11c[b]	1.81 (s, 3H, $NHCOCH_3$); 3.06 (m, 2H, Arg δ-CH_2); 3.22 (m, 2H, Trp β-CH_2); 3.40 (s, 3H, CO_2CH_3); 4.27 (m, 1H, Arg α-CH); 4.45 (m, 1H, Trp α-CH); 6.68 (dd, 1H, Nps H-6); 8.28 (dd, 1H, Nps H-3); 8.59 (d, 1H, CONH).
11d[b,d]	1.81 (s, 3H, $NHCOCH_3$); 2.97 (m, 2H, Arg δ-CH_2); 3.23 (m, 2H, Trp β-CH_2); 3.47 (s, 3H, CO_2CH_3); 4.24 (m, 1H, Arg α-CH); 4.51 (m, 1H, Trp α-CH); 6.66 (dd, 1H, Nps H-6); 8.28 (dd, 1H, Nps H-3); 8.47 (d, 1H, CONH).
12c+12d[e]	1.93 and 1.95 (2s, 3H, intensity ratio 8:3, $NHCOCH_3$); 2.88 and 3.20 (2m, 2H, intensity ratio 3:8, D- and L- Arg δ-CH_2); 3.30 and 3.31 (2m, 2H, intensity ratio 3:8, Trp β-CH_2); 3.71 and 3.77 (2s, 3H, intensity ratio 8:3, CO_2CH_3); 4.04 and 4.15 (2m, 1H, D- and L-Arg α-CH); 4.68 (m, 1H, Trp α-CH).
16a[a]	2.16 [s, 6H, $N(CH_3)_2$]; 3.70 (s, 3H, CO_2CH_3); 4.83 (m, 1H, Trp α-CH); 5.10 (s, 2H, ZCH_2); 7.33 (s, 5H, ZC_6H_5).
16b[a]	2.10 [s, 6H, $N(CH_3)_2$]; 3.70 (s, 3H, CO_2CH_3); 4.90 (m, 1H, Trp α-CH); 5.03 (s, 2H, ZCH_2); 7.30 (s, 5H, ZC_6H_5).
17a[a]	2.28 [s, 6H, $N(CH_3)_2$]; 3.70 (s, 3H, CO_2CH_3); 4.93 (m, 1H, Trp α-CH); 5.02 (s, 2H, ZCH_2); 6.67 (dd, 1H, Nps H-6); 7.30 (s, 5H, ZC_6H_5); 8.20 (dd, 1H, Nps H-3).

Table 3.: Continued

No.	δ(ppm)
17b·HCl[a]	2.43 [s, 6H, $N(CH_3)_2$]; 3.60 (s, 3H, CO_2CH_3); 4.92 (m, 1H, Trp α-CH); 5.03 (s, 2H, ZCH_2); 6.73 (dd, 1H, Nps H-6); 7.23 (s, 5H, ZC_6H_5); 8.15 (dd, 1H, Nps H-3).
18a[b]	2.11 [s, 6H, $N(CH_3)_2$]; 2.62 (m, 2H, Lys ε-CH_2); 2.89 (m, 1H, Lys α-CH) 3.27 (m, 2H, Trp β-CH_2); 3.53 (s, 3H, CO_2CH_3); 4.63 (m, 1H, Trp α-CH); 6.68 (dd, 1H, Nps H-6); 8.31 (dd, 1H, Nps H-3).
18b·HCl[b]	2.31 and 2.57 [2s, 6H, $N(CH_3)_2$]; 2.80 (m, 2H, Orn δ-CH_2); 3.30 (m, 2H, Trp β-CH_2); 3.58 (s, 3H, CO_2CH_3); 3.73 (m, 1H, Orn α-CH); 4.48 (m, 1H, Trp α-CH); 6.68 (dd, 1H, Nps H-6); 8.30 (dd, 1H, Nps H-3).
18c·2HCl[b]	2.37 and 2.52 [2s, 6H, $N(CH_3)_2$]; 3.31(m, 2H, Trp β-CH_2); 3.50 (m, 2H, Arg δ-CH_2); 3.53 (s, 3H, CO_2CH_3); 3.76 (m, 1H, Arg α-CH); 4.79 (m, 1H, Trp α-CH); 6.69 (dd, 1H, Nps H-6); 8.30 (dd, 1H, Nps H-3).

a) In $CDCl_3$ at 90 MHz; b) in DMSO-d_6 at 300 MHz; c) in DMSO-d_6 at 90 MHz. d) Data obtained from the spectrum of the 8:3 mixture of **11c+11d**. e) In D_2O at 300 MHz.

Table 4. Analgesic response to the N-Acetyl Dipeptides Derivatives **11a-d** in the Tail-Flick Test in Mice

Compd.	Dose, μg/mouse icv	% Change in reaction time (min)[a] 5	30	60
Saline		9±8	7±5	5±9
11a	0.50	96±24*	60±19*	34±15
	1.00	150±22*	90±22*	30±12
11b	0.25	90±30*	59±22*	-1±10
	0.50	b	b	b
11c+11d	0.25	99±25*	48±17*	15±7
(c:d, 3:1)	0.50	b	b	b
2a	0.50	98±21*	50±16*	20±7
	1.00	140±23*	73±11*	45±6*
2b	0.50	125±28*	42±10*	20±10
2c	0.50	120±18*	52±10*	22±5

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

Experimental Part
Chemical methods

Mp. (uncorrected): Kofler hot-stage apparatus.-Elemental analyses: Heraeus CHN-O-RAPID instrument.-UV-spectra: Perkin Elmer 550 spectrophotometer. – ^{1}H-NMR spectra: Varian EM-390 (90 MHz) and Varian XL-300, TMS int. stand. -Analytical TLC: Aluminium sheets coated with a 0.2mm layer silica gel (60 F_{254}, Merck).-Column chromatography: silica gel (60, 230-400 mesh, Merck). Compounds were detected with UV light (254 nm) and ninhydrin spray.

All the amino acids used were of the L-configuration unless otherwise specified. Z-δApn-OH was prepared as described[17]. Z-εAhx-OH, H-Trp-OMe·HCl and Ac-Arg-OH·HCl were purchased from Bachem, Nps-Cl was from Sigma.

N^{α}, N^{α}-Dimethyl-N^{ε}-(benzyloxycarbonyl)lysine (**14a**)

2.0 g (7.1 mmol) H-Lys(Z)-OH and 7.2 mL 35 % aqueous formaldehyde in 22 mL MeOH were refluxed for 30 min. Then the solution was cooled to room temp. and 0.95 g (24 mmol) $NaBH_4$ were added. After stirring for 15 h, the solvents were evaporated i.vac. and the residue was dissolved in 20 mL H_2O and neutralized with conc. HCl. The aqueous solution was evaporated and the residue was treated with EtOH. After filtration, the filtrate was evaporated to dryness and the residue was purified by column chromatography (cc) using $CHCl_3$: MeOH 2:1 as eluent: 1.8 g (82%), mp 148-150 °C (acetone). -$C_{16}H_{24}N_2O_4$ (280.3) Calc. C 62.3 H 7.84 N 9.1. Found C 62.0 H 8.04 N 8.8.-^{1}H-NMR (DMSO-d_6, 90 MHz): δ1.20-2.00 (m, 6H, Lys β-, γ- and δ-CH_2), 2.75 [s, 6H, $N(CH_3)_2$)], 3.00 (m, 2H, Lys ε-CH_2), 3.90 (m, 1H, Lys α-CH), 5.00 (s, 2H, ZCH_2), 7.33 (s, 5H, ZC_6H_5).

*N^{α},N^{α}-Dimethyl-N^{δ}-(benzyloxycarbonyl)ornithine (**14b**) and N^{α}-hydroxymethyl-N^{α}-methyl-N^{α}-(benzyloxycarbonyl) ornithine (**15b**)*

2.5 g (9.4 mmol) H-Orn (Z)-OH and 14 mL 35% aqueous formaldehyde in 30 mL MeOH were refluxed for 30 min. After cooling to room temp., the solution was treated with 1.21 g (32 mmol) $NaBH_4$ for 15 h, and the reaction mixture was worked up and purified as described for **14a** to give 2.61 g of a gummy solid which was identified by its ^{1}H-NMRspectrum as a 1:3 mixture of **14b** and **15b**: ^{1}H-NMR of **15b** (DMSO-d_6, 300 MHz): δ 1.50-1.90 (m, 4H, Orn β-and γ-CH_2), 2.24 [s, 3H, $N(CH_3)$], 2.98 (m, 2H, Orn δ-CH_2), 3.23 (m, 1H, Orn α-CH), 4.30 and 4.52 (2d, J=14.0 Hz, 2H, $C\underline{H}_2OH$), 5.07 (s, 2H, ZCH_2), 7.36 (s, 5H, ZC_6H_5).

The above mixture in 40 mL MeOH reacted with 2.42 g (64 mmol) $NaBH_4$ at room temp. for 15 h. Then it was neutralized with conc. HCl and the solvent removed. The resulting residue was treated with 75 mL EtOH, filtered and the filtrate evaporated to dryness to give a solid which was purified by cc using $CHCl_3$: MeOH 3:1 as eluent: 2.17 g (58.5% from H-Orn(Z)-OH), mp. 142-144 °C (acetone).-$C_{15}H_{22}N_2O_4$ (294.3) C 61.2 H 7.53 N 9.5. Found C 60.9 H 7.81 N 9.2. -^{1}H-NMR(DMSO-d_6, 90 MHz): δ 1.30-1.90 (m, 4H, Orn β-and γ-CH_2), 2.76 [s, 6H, $N(CH_3)_2$)], 3.06 (m, 2, Orn δ-CH_2), 3.98 (m, 1 H, Orn α-CH), 5.00 (s, 2H, ZCH_2), 7.33 (s, 5H, ZCH_6H_5).

Coupling reactions using the DCC/HOSu method. (General procedure for **4, 12** *and* **(16)**

Equimol. amounts (4 mmol) of the corresponding starting amino acid derivative (**3**, Ac-Arg-OH·HCl and **14**), N-Hydroxysuccinimide and DCC in 40 mL of the appropiate solvent (THF for **3**; 1,2-dimethoxyethane for **14a**, and DMF for Ac-Arg-OH·HCl and **14b**) were stirred at room temp. When the complete formation of the N-hydroxysuccinimide ester was detected by tlc, equimol. amounts of H-Trp-OMe·HCl and Et_3N were added and stirring was continued for 1 h (comp. **4**) or 15 h (comp. **12** and **16**). The solvent was removed i.vac. and the residue was purified by cc using the following eluents: EtOAc: hexane 2:1 (**4a**); $CHCl_3$: MeOH 12:1 (**4b**); $CHCl_3$: MeOH 6:1 (**12a+12b**); EtOAc: acetone 3:1 (**16a**), and $CHCl_3$: MeOH 9:1 (**16b**). Analytical and spectral data are listed in tables 1 and 3.

Nps-Lys(Z)-Trp-OMe **(8a)**

2.04 g (8 mmol) H-Trp-OMe·HCl in 20 mL dry THF were treated with 1.1 mL (8 mmol) Et_3N and the resulting salt was removed by filtration. Then 3.67 g (8 mmol) **7a**[10] was added to the filtrate and the mixture was stirred at room temp. for 2 h. The solvent was evaporated, the residue dissolved in 40 mL EtOAc, and the solution was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O. The org. phase was dried (Na_2SO_4) and evaporated. Analytical and spectral data are listed in tables 1 and 3.

Addition of Nps-Cl to Trp-containing dipeptide analogues. (General procedure for **5, 11c+11d** *and* **17)**

N-Protected dipeptide methyl esters (2 mmol), **4, 12c+12d** and **16a** in 40 mL 1N HCl/dioxane or **16b** in 40 mL N HCl/MeOH were reacted with Nps-Cl (2.2 mmol) at room temp. for 1 h. The solvents were removed i.vac. and the residue was purified by cc using EtOAc: hexane 2:1 for **5**, $CHCl_3$: MeOH 6:1 for **11c+11d** and $CHCl_3$: MeOH 9:1 for **17**, respectively. Compound **17b** was subsequently converted into its hydrochloride. Analytical and spectral data of all these Trp(Nps)-containing dipeptides are recorded in tables 1 and 3.

H-Lys(Z)-Trp(Nps)-OMe·HCl **(9a)**

3.5 g (5.5 mmol) **8a** was dissolved in 15 mL N HCl/dioxane. The solution was kept at room temp. for 30 min, evaporated and the residue was triturated with benzene. Analytical and spectral data are listed in tables 1 and 3.

Ac-Lys(Z)-Trp(Nps)-OMe **(10a)**

0.3 g (0.5 mmol) **9a** and 0.061 g (0.5 mmol) 4-dimethylaminopyridine in 5 mL $(AcO)_2O$ were stirred at room temp. for 30 min. Then, the reaction mixture was poured into 150 mL iced H_2O and extracted with $CHCl_3$. The org. phase was dried (Na_2SO_4), evaporated and the residue crystallized. Analytical and spectral data are listed in tables 1 and 3.

Ac-Orn(Z)-Trp(Nps)-OMe **(10b)**

0.66 g (1 mmol) **9b**[9] and 0.12 g (1 mmol) 4-dimethylaminopyridine in 8 mL $(AcO)_2O$ were stirred at room temp. for 1 h, and then 30 mL ether were added. The precipitate was filtered and purified by cc using $CHCl_3$: MeOH 9:1 as eluent. Analytical and spectroscopic data are recorded in tables 1 and 3.

Ac-Arg-Trp(Nps)-OMe·HCl **(11c)**

0.3 g (0.47 mmol) H-Arg-Trp(Nps)-OMe·2HCl[1] in 5 mL DMF reacted with 0.05 mL (0.47 mmol) acetyl chloride in the presence of 0.13 mL (0.94 mmol) Et_3N. After 30 min, the solvents were removed and the residue chromatographed on a silica gel column using $CHCl_3$:MeOH 9:1: **11c** and Et_3N·HCl. Al attempts to obtain **11c** free from Et_3N·HCl were unsuccessful. The ^{1}H-NMR spectrum of **11c** is listed in table 3.

Removal of the benzyloxycarbonyl protecting group with boron-tris(trifluoroacetate)/trifluoracetic acid. (General procedure for **6a,b** *and* **11a)**

BTFA in TFA (3 equiv) was added to a cooled solution (0 °C) of the corresponding Z protected dipeptide analogue (1.5 mmol) in 3 mL TFA and the mixture was stirred at room temp. for 20 h. The solvent was removed and the residue purified by cc using $CHCl_3$: MeOH 9:1 for **6a** and **11a**, and $CHCl_3$: MeOH 7:2 for **6b**. Analytical and spectroscopic data are listed in tables 2 and 3.

Removal of the benzyloxycarbonyl protecting group with trimethylsilyl iodide. (General procedure for **11b** *and* **18a,b)**

0.15 mL Me_3SiI (1 mmol) was added to the corresponding Z protected dipeptide (0.6 mmol) in 7 mL dry acetonitrile, and the mixture was stirred for 10 min at room temp. After this, 7 mL MeOH were added, the solvents were removed and the residue purified by cc using $CHCl_3$: MeOH 6:1. Compound **18b** was subsequently converted into its hydrochloride. Analytical and spectroscopic data are listed in tables 2 and 3.

Guanidylation method. (General procedure for **6c** *and* **18c)**

Orn-containing dipeptide (0.53 mol) and ADMPN (0.6 mmol) in 10 mL THF and 0.1 mL Et_3N were refluxed for 4 h. The solvent was removed and the residue purified by cc using $CHCl_3$: MeOH 3:1 for **6c** and $CHCl_3$: MeOH 6:1 for **18c**. Compound **18c** was subsequently transformed into its dihydrochloride. Analytical and spectroscopic data are listed in tables 2 and 3.

Analgesic 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 *Notti*[18] 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 reaction time was in the range of 1.8-2 s. The peptides were dissolved in 0.01 N HCl, neutralized with 0.01 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.

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5 Symbols and abbreviations are in accordance with the recommendations made in 1983 by the IUPAC-IUB Commission, published in the Biochem. J. *219*, 345 (1984). εAhx, δApn and δGpn are 6-aminohexanoic acid, 5-aminopentanoic acid and 5-guanidinopentanoic acid, respectively. NCA means N-carboxy-α-aminoacid anhydride. The prefix *ambo* is used in the case where a mixture of diastereomers exists. Thus, Ac-*ambo*-Arg-Trp(Nps)-OMe signifies a mixture of Ac-L-Arg-Trp(Nps)-OMe and Ac-D-Arg-Trp(Nps)-OMe. -cc: column chromatography.

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