at 0°C in the presence of anisole; working up was with ether), the presence of a spot with lower R_f value was observed indicating removal of the ONp group had also taken place here.

H-Phe-Met-εAhx-OH

Boc-Phe-Met-εAhx-OH was treated with TFA/H_2O (9/1, v/v) in the presence of anisole for 1 h at room temperature. The solution was then concentrated and ether added. The isolated oily product was dissolved in *tert*-butanol/water and the solution treated with an ion-exchange resin in the acetate form, and lyophilized. The peptide was freed from traces of by-products by chromatography on silica gel using solvent system g.
$[\alpha]_D^{21}$ +11.8° (*c* 1, 10% aqueous HOAc). TLC: R_f 0.70 (g), 0.37 (d). Amino acid analysis: Phe 1.03, Met 1.04, εAhx 0.94. LAO digestion: no racemization of Phe and Met was found.
HPLC: main component 94% (mobile phase as in ref. 15; 25 min linear gradient of 100%A–0%B to 30%A–70%B and then 5 min at this composition).

Cyclo(*-Phe-Met-εAhx-*)

a.1 *via H-Phe-Met-εAhx-ONp·HCl*
H-Phe-Met-εAhx-ONp·HCl was dissolved in a small volume of DMF. This solution was added dropwise over a period of 5 h (with stirring) to pyridine (approx. 600 mg peptide/l pyridine) at about 50°C under a N_2 blanket. After standing overnight the solution was evaporated to dryness, the residue dissolved in MeOH and the solution evaporated again. TLC showed that the active ester had disappeared but that the lower-running fluorescamine-positive H-Phe-Met-εAhx-OH was still present.
Trituration of the residue with water and subsequent filtration was performed several times; this treatment removed the fluorescamine-positive by-products. Further washing with methanol gave the title compound in approx. 47% yield. TLC of the sparingly soluble cyclic peptide (a 0.5% solution in warm DMF or trifluoroethanol was used) showed a nearly homogeneous preparation; R_f 0.92 (b), 0.85 (d), 0.60 (i). M.p. >270°C. Amino acid analysis: Phe 0.98, Met 0.96, εAhx 1.08. LAO digestion: no racemization of Phe and Met was found. Mass spectrometry: M^+ peak 391; no di- or trimeric isomers were found. ^1H NMR spectroscopy of a dilute solution in $CDCl_3$ + 10% CD_3OD: δ 2.0 (s, CH_3S–); δ 3.0 (AB part of ABX system, $-CH_2-Ph$); δ 4.5 (t, X part of ABX system, $-CH-CH_2-Ph$); δ ca. 7.3 (m, C_6H_5–).

a.2 *via H-Phe-Met-εAhx-ONp·TFA*
Cyclization of H-Phe-Met-εAhx-ONp·TFA as described under *a*.1 gave, after recrystallization from warm DMF/ether, the cyclic peptide in a yield of approx. 34%. TLC: R_f 0.63 (a). Analytical data were in agreement with those reported under *a*. **1**.

b. via H-Phe-Met-εAhx-OH
The free peptide was dissolved in DMF (approx. 1.5 g/l) and the solution cooled to approx. −20°C. The pH was adjusted to 7.5 with Et_3N and 4 ml of DPPA (Aldrich) were added. After one day at −20°C the reaction mixture was placed at 0°C and kept for 4 more days. On days 2 and 4, 1 ml of DPPA was added while the pH was maintained at approx. 7.5 by periodic addition of triethylamine.
After 5 days, no fluorescamine-positive material could be detected on TLC plates; the reaction mixture was concentrated to about one-third of its volume and water was added. All ionic species were then removed by treatment with a mixture of acid and base ion-exchange resins. After filtration, the filtrate was evaporated to dryness and the residue triturated with methanol; yields ranged from 30 to 70%. For further purification the material was chromatographed on a silica gel column (Merck Fertigsäule) using the solvent system chloroform/trifluoroethanol (4/1, v/v); overall yields 10 to 20%. TLC showed the same R_f values as given under *a*. **1**. Correct amino acid compositions and mass spectra were obtained; no racemization was found.

Acknowledgements

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Synthesis of peptide-morphinans based on *Diels–Alder* adducts of thebaine with enkephalin moieties (Chemistry of opium alkaloids, Part XVI)*

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Abstract. The preparation of *Diels–Alder* adducts of (−)-thebaine and ethyl acrylate is described. Hydrolysis of the major adduct gave the 7α-carboxylic acid which was coupled with the ethyl esters of L-leucine, L-phenylalanyl-L-leucine and glycyl-L-phenylalanyl-L-leucine, respectively, three peptide segments derived from the endogenous opiate, leucine-enkephalin. These compounds, as well as the 7β-ethoxycarbonyl isomer, were *O*-demethylated to give the corresponding 3,6-dihydroxymorphinan derivatives. *N*-(6,14-*endo*-Etheno-6,7,8,14-tetrahydrothebaine-7α-carbonyl)-L-phenylalanyl-L-leucine ethyl ester and its 7,8-dihydromorphine analogue were reduced to the corresponding leucinols.
Pharmacological screening** showed that several of these compounds are morphine-like analgesics, notably compound **11**, *N*-(6,14-*endo*-etheno-7,8-dihydromorphine-7α-carbonyl)-L-leucine ethyl ester.

Introduction

The identification of methionine- and leucine-enkephalin, two brain pentapeptides with opiate activity, by *Hughes*, *Kosterlitz* et al.[1] has intensified the study of the structure–activity relationships of analgesic compounds. A struc-

* Part XV: *P. R. Crabbendam*, *L. Maat* and *H. C. Beyerman*, Recl. Trav. Chim. Pays-Bas **100**, 293 (1981).
** Dr. *A. E. Jacobson*, National Institutes of Health, Bethesda, Maryland, U.S.A.
[1a] *J. Hughes*, Brain Res. **88**, 295 (1975).
[b] *J. Hughes*, *T. W. Smith*, *H. W. Kosterlitz*, *L. A. Fothergill*, *B. A. Morgan* and *H. R. Morris*, Nature (London) **258**, 577 (1975).

tural comparison[2] with morphine, the "classical" centrally acting analgesic, shows that the tyrosine moiety is common to both molecules (Fig. 1). Enkephalin should be able to assume various conformations in aqueous solution and several preferred conformations have been suggested[3-5]. Investigations of leucine-enkephalin, having an azo bridge connecting the two aromatic rings[6], indicated one predominant conformation[4]. From biological screening of several analogues[7], models of the interactions between enkephalin and its receptor have been proposed.

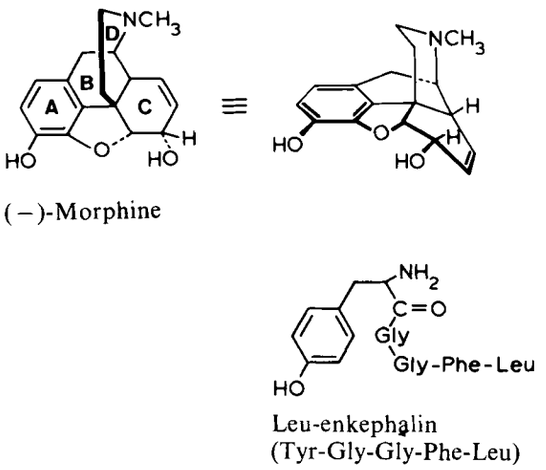


Fig. 1. Comparison of tyrosine part, both in (−)-morphine and leucine-enkephalin.

There is some similarity between the "tail" of the enkephalins and the lipophilic part of the *Diels–Alder* modified morphinans described by *Bentley* et al.[8]. The latter compounds are highly potent analgesics, prepared by addition of dienophiles, *e.g.* ethyl acrylate, to (−)-thebaine, followed by conversion of the ester group in position 7α into a lipophilic "tail". A well-known example of such a compound is etorphine with the (*R*)-configuration at C(19)[9] (Fig. 2).

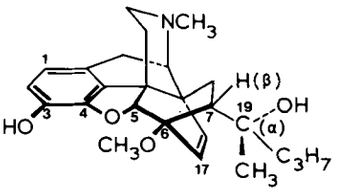
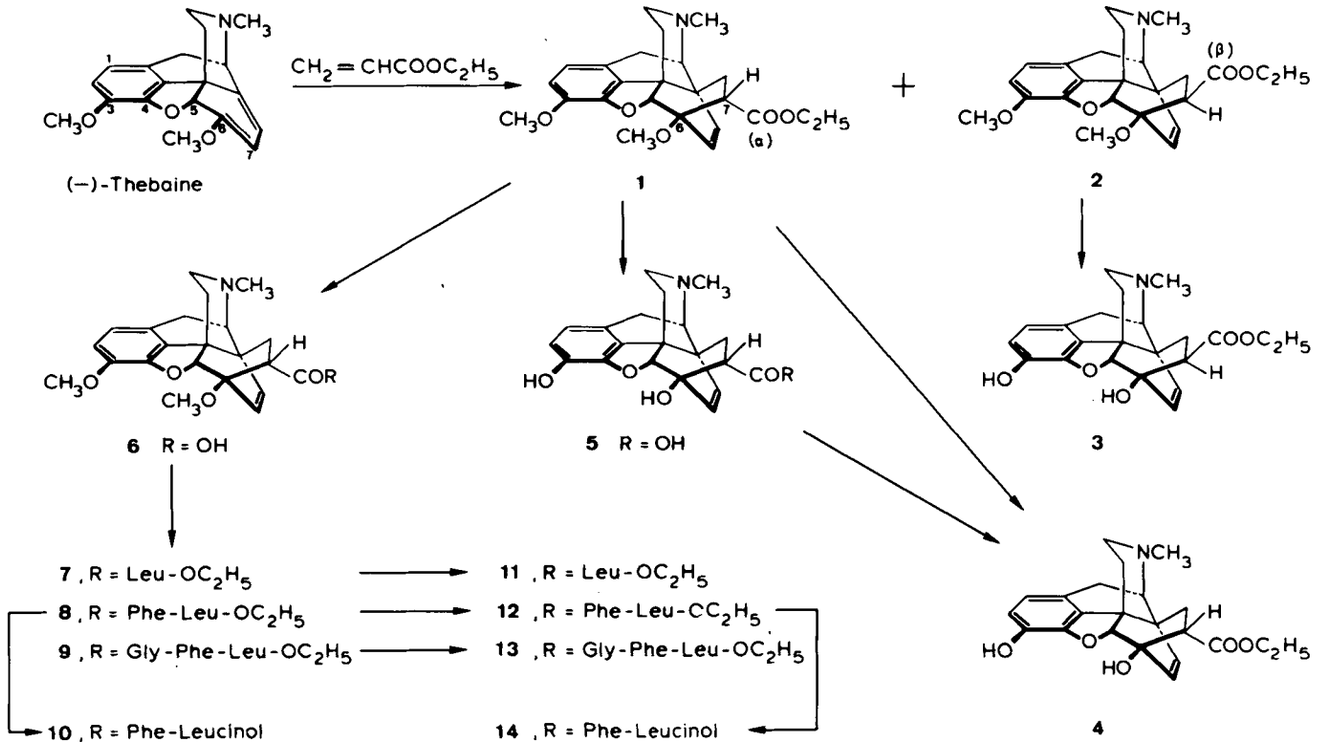


Fig. 2. Etorphine.

[2a] *A. F. Bradbury, D. G. Smyth* and *C. R. Snell*, Nature (London) **260**, 165 (1976).
[b] *A. S. Horn* and *J. R. Rodgers*, Nature (London) **260**, 795 (1976).
[3] *F. A. Gorin* and *G. R. Marshall*, Proc. Natl. Acad. Sci. USA **74**, 5179 (1977).
[4] *G. D. Smith* and *J. F. Griffin*, Science **199**, 1214 (1978).
[5] *H. Kessler* and *G. Hölzemann*, Justus Liebigs Ann. Chem. 2028 (1981).
[6a] *I. Z. Siemion, Z. Szewczuk, Z. S. Herman* and *Z. Stachura*, Abstracts IUPAC 12th International Symposium on the Chemistry of Natural Products, Puerto de la Cruz, Spain, Sept. 12–27, 1980, A21.
[b] *I. Z. Siemion, Z. Szewczuk, Z. S. Herman* and *Z. Stachura*, Mol. Cell. Biochem. **34**, 23 (1981).
[7] *D. Hudson, R. Sharpe* and *M. Szelke*, Int. J. Pept. Protein Res. **15**, 122 (1980).
[8] *J. W. Lewis, K. W. Bentley* and *A. Cowan*, Annu. Rev. Pharmacol. **11**, 241 (1971).
[9] The compounds are named and numbered according to *Bentley* (refs. 8 and 11) and many other authors. This deviates from Chemical Abstracts Service (CAS) usage. CAS denominates the ring system 6,14-ethenomorphinan. In natural morphinans, the B and C rings are *cis*-fused. This is also depicted in IUPAC Rule F-4.12 (Example 46) for the morphinan ring system. A systematic name for the new ring system, therefore, would be 7,8-didehydro-6,14-ethanomorphinan.
Furthermore, CAS designates the ring nitrogen atom by number 17, as a consequence of which the two carbon atoms of the ethano-bridge must have the numbers 18 and 19. The first carbon atom outside the ring system at C(18) has the description "α" in the CAS nomenclature and the number 19 in the "Bentley nomenclature".



Scheme. Diels–Alder *adducts of thebaine and coupling products with enkephalin moieties.*

In our search for analgesic compounds, having greater selectivity and fewer undesirable side-effects and based on naturally occurring structures, we synthesized compounds which combine a morphinan part with the *C*-terminal residue of an enkephalin. For this purpose, ethyl acrylate was added to (−)-thebaine. Coupling of the acid group, obtained by hydrolysis of the ester at position 7, with a residue of leucine-enkephalin, resulted in a molecule which retains the rigid ring system of the "Bentley adducts" and also possesses the lipophilic part of the enkephalins. *O*-Demethylation at position 3 yielded compounds which resemble the tyrosine part of enkephalin and the A-ring of morphine. At the C-ring, our compounds have the amide bond of C(19) in the 7α position which results in more restricted rotation compared to that observed in the compounds studied by *Bentley*. In the case of the compounds with a hydroxyl group at position 6, hydrogen bond formation seems possible. This might influence the conformation of the lipophilic part via C(19)[10]. The 7β-isomers, which were obtained in low yield, will be the subject of further study.

Results and discussion

In order to effect the coupling of a morphinan with L-leucine ethyl ester and the enkephalin segments, we used the reaction products of (−)-thebaine with ethyl acrylate (Scheme). The *Diels–Alder* addition yielded two isomers. The main compound (95%) was ethyl 6,14-*endo*-etheno--6,7,8,14-tetrahydrothebaine-7α-carboxylate (**1**) and a minor compound (5%), the 7β-isomer (**2**), was isolated from the mother liquor. The ester **1** was hydrolyzed with sodium hydroxide in dioxane, which proved to be advantageous compared to acidic hydrolysis[11], to give 6,14-*endo*--etheno-6,7,8,14-tetrahydrothebaine-7α-carboxylic acid (**6**). The best conditions found for coupling with the ethyl ester of leucine and the peptides involved the intermediacy of the acid chloride of **6**, which was easily prepared by reaction of **6** with oxalyl chloride. In early experiments, the coupling was carried out by means of the excess mixed anhydride (EMA) method using isobutyl chloroformate, a standard method in peptide chemistry. Although the desired product was obtained, a large amount of a by--product was also formed due to reaction of the isobutoxycarbonyl residue with the amino component of the peptide. This side-reaction is known to occur with sterically hindered amino acids[12]; the amino substituent reacts with the undesired part of the mixed anhydride.

Reaction of the freshly prepared acid chloride of **6** with, respectively, the ethyl ester of L-leucine, L-phenylalanyl-L--leucine and glycyl-phenylalanyl-L-leucine yielded, almost quantitatively, the compounds 7, **8** and **9**.

The reduction of the *C*-terminal acid of synthetic enkephalin analogues to the alcohol function caused, in a number of cases, a considerable increase in the enkephalin potency[13]. Thus, the ester group of **8** and **12** was selectively reduced with sodium tetrahydroborate and calcium chloride in 2-propanol to give **10** and **14**, respectively. An excess of calcium chloride suppresses the partial hydrolysis of the sensitive ester[14].

In many morphinans, opiate potency is increased upon demethylation of the 3-methoxy substituent, *e.g.* codeine to morphine. This effect is also found for the *Diels–Alder* reaction products[15]. In agreement with this is the dramatic decrease in morphine-like activity of enkephalin derivatives upon methylation of the hydroxyl group of the tyrosine moiety[13]. The hydroxyl group in position 3, however, may not be necessary since 4-hydroxylated 3-deoxymorphinan compounds also show morphine-like activity[16].

Efforts to selectively demethylate the 3-methoxyl group, while maintaining it in position 6, were unsuccessful. Only the 3,6-demethylated products, therefore, were prepared. For the preparation of these morphinan peptides, three approaches were used: *(i)* coupling of the 3,6-hydroxy compound **5** with L-leucine ethyl ester or with the peptides; *(ii)* temporary blocking of the two hydroxyl groups of **5** followed by coupling; and *(iii)* 3,6-demethylation of the coupling products **7**, **8** and **9**.

(i) In the first approach, **1** was demethylated. Careful treatment with hydrogen bromide in glacial acetic acid split the methoxy groups but also caused partial hydrolysis of the ester. Refluxing the crude reaction product with ethanol and hydrogen chloride afforded the ester **4**. In contrast, treatment with concentrated hydrogen bromide not only demethylated the methoxy groups but also hydrolyzed the ester to the carboxylic acid **5**. Preliminary determination of the structure of **5** involved conversion into the ester **4**. The coupling of **5** with the peptides, using the excess mixed anhydride method, proved to be incomplete and gave many by-products. *Woodward*'s reagent K, known as a coupling agent for compounds with unprotected hydroxyl groups (*e.g.* tyrosine, serine and threonine)[17], was also unsuccessful.

(ii) For temporary protection of the hydroxyl groups, the pyranyl and benzyl ether and the acetate ester substituents were examined. Protection via the diacetate seemed to be most promising, however success using the third approach led to the abandonment of this route.

(iii) Demethylation of the coupled products **7**, **8** and **9** proved to be feasible using hydrogen bromide in glacial acetic acid. The carboxylic acid, formed by partial hydrolysis of the ester, was re-esterified by treatment with hydrogen chloride in ethanol. In this way, **11** was obtained from compound **7**, and **8** and **9** were obtained from **12** and **13**, respectively. For the reasons mentioned above, the ester group of **12** was reduced with sodium tetrahydroborate and calcium chloride to give the alcohol **14**. The demethylated β-isomer **3** was obtained from **2** as described for the analogous α-isomers.

Pharmacology**

Some of the compounds were potent antinociceptives in the mouse hot plate assay (sc injection). The pharmacological activity of compound **11** was also determined in the opiate receptor assay in rat brain membranes, in electrically stimulated guinea pig ileum and in mouse vas deferens preparations. Compound **11** also proved to be

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[10] *G. H. Loew* and *D. S. Berkowitz*, J. Med. Chem. **22**, 603 (1979).

[11] *K. W. Bentley* and *D. G. Hardy*, J. Am. Chem. Soc. **89**, 3281 (1967).

[12] *H. C. Beyerman*, "Chemistry and Biology of Peptides", *G. Meienhofer* (Editor), Ann Arbor Science Publ., Ann Arbor, Michigan (1972), 6351.

[13] *H. Moritoki*, *Y. Kiso*, *T. Kageyama* and *K. Matsumoto*, J. Pharm. Pharmacol. **33**, 54 (1981).

[14] *H. C. Beyerman*, *A. W. Buijen van Weelderen*, *L. Maat* and *A. Noordam*, Recl. Trav. Chim. Pays-Bas **96**, 192 (1977).

[15] *K. W. Bentley*, *J. D. Bower* and *J. W. Lewis*, J. Chem. Soc. C, 2569 (1969).

[16] *A. E. Jacobson*, *F. L. Hsu*, *M. D. Rozwadowska*, *H. Schmidhammer*, *L. Atwell*, *A. Brossi* and *F. Medzihradsky*, Helv. Chim. Acta **64**, 1298 (1981).

[17] *Y. S. Klausner* and *M. Bodansky*, Synthesis 459 (1972).

quite potent in these tests. Compound **11** displays an unusual characteristic as a displacer of etorphine in the binding assay. In the smooth muscle preparations, **11** is somewhat more potent than morphine and its pattern of interaction with two antagonists led us to consider it morphine-like.
Details of these experiments and of further studies will be published elsewhere.

Experimental

Mass spectra were measured by Dr. *P. J. W. Schuyl* and Mrs. *A. H. Knol-Kalkman* using a Varian-Mat SM-1 mass spectrometer. ^{1}H NMR spectra were measured using a Varian T-60 spectrometer. The compounds were dissolved (10% w/v) in deuteriochloroform, hexadeuteriodimethyl sulfoxide or deuterium oxide. Infrared spectra were obtained from KBr discs using a Beckman IR 4210 spectrophotometer. Analytical HPLC was performed using a Waters M-6000 pump on a reverse-phase column (15 cm × 0.4 cm I.D., Nucleosil C_{18}, 7 µm, 30°C) with isocratic mixtures of methanol and water, with detection on a Pye LC 3 variable wave length detector at 240–250 nm. TLC was performed on deactivated silica gel (Merck F-254) with dichloromethane/methanol/2 *N* ammonia 85 : 15 : 2 as the mobile phase. The compounds were detected with UV (254 nm) and iodine vapour. Fluorescamine was used for primary amino group detection. Combustion analyses were performed by Mr. *H. M. A. Buurmans* and the Element Analytical Section of TNO, Utrecht (supervision by Mr. *G. J. Rotscheid*). Melting points are uncorrected. Optical rotations were measured using a Perkin-Elmer Polarimeter P 141.

N-*(*tert*-Butoxycarbonyl)*-L-*phenylalanyl*-L-*leucine ethyl ester*

To a solution of 18.55 g (70 mmol) of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine and 7.8 ml (70 mmol) of *N*-methylmorpholine (NMM) in 40 ml of dimethylformamide (DMF) was added, at −15°C, 8.65 ml (65 mmol) of isobutyl chloroformate. After 2 min, a cooled solution (−15°C) of 9.8 g (50 mmol) of L-leucine ethyl ester·HCl[18] in 20 ml of DMF and 5.55 ml (50 mmol) of NMM was added. The pH was adjusted to 7.5 with some NMM. After 15 min, the reaction with fluorescamine was negative. After a further 20 min at −15°C, 70 ml of 2 M potassium hydrogen carbonate was added dropwise. The temperature was raised to 0°C and 20 ml of DMF was added. The mixture was stirred for 30 min 300 ml of water was then added and stirring continued for a further 30 min at 0° C. After 40 min at 4° C, the precipitate was filtered and washed with 100 ml of 0.2 M potassium hydrogen carbonate, 100 ml of 0.1 M potassium hydrogen carbonate, 100 ml of water and 50 ml of water, respectively. *N*-(*tert*-Butoxycarbonyl(-L-phenylalanyl-L-leucine ethyl ester [20.2 g, 50 mmol, 99%, pure according to TLC (chloroform/methanol 4 : 1)] was recrystallized from ethanol/petroleum ether; m.p. 117–118°C and $[\alpha]_D^{25}$ −21.7° (*c* 2.4, ethanol).

N-*(*tert*-Butoxylcarbonyl)glycyl*-L-*phenylalanyl*-L-*leucine ethyl ester*

To a solution of 9.6 g (55 mmol) of *N*-(*tert*-butoxycarbonyl)-glycine and 6.2 ml (55 mmol) of NMM in 35 ml of DMF was added, at −15°C, 6.9 ml (52 mmol) of isobutyl chloroformate. After 2 min, a cooled solution (−15°C) of 37 mmol of L-phenylalanyl-L-leucine ethyl ester and 4.2 ml (37 mmol) of NMM in 60 ml of DMF was added. L-Phenylalanyl-L-leucine ethyl ester was obtained from the corresponding *N*-(*tert*-butoxycarbonyl)-L-phenylalanyl-L-leucine ethyl ester in the usual way by treatment with trifluoroacetic acid and dichloromethane (1 : 1). The pH was adjusted to 8 with some NMM. When the reaction with fluorescamine became negative, the mixture was stirred for about 60 min at −15°C. The temperature was raised to 0°C, 53 ml of 2 M potassium hydrogen carbonate was added dropwise and stirring continued for a further 30 min. The reaction mixture was then poured into 250 ml of water and extracted with ethyl acetate. The organic layer was washed with 0.5 M potassium hydrogen carbonate and water, respectively, until neutral, and dried over magnesium sulfate. The solvent was evaporated *in vacuo* to yield *N*-(*tert*-butoxycarbonyl)glycyl-L-phenylalanyl-L-leucine ethyl ester (15.6 g, 91%); m.p. 104–107°C; $[\alpha]_D^{25}$ −22.1° (*c* 1.0, ethanol). Calcd. for $C_{24}H_{37}N_3O_6$ (463.56): C 62.18, H 8.05, N 9.07; found: C 62.1, H 8.1, N 9.1.

Ethyl 6,14-endo-*etheno-6,7,8,14-tetrahydrothebaine-7α-carboxylate* (**1**)

Thebaine (109 g, 0.35 mol) and 190 ml (1.75 mol) of freshly distilled ethyl acrylate was boiled under reflux for 5½ h. The excess ethyl acrylate was removed *in vacuo* with the addition of some ethanol. The residue was taken up in 900 ml of warm ethanol and the fine precipitate formed was removed. After standing overnight at room temperature, the mixture was filtered and the collected precipitate washed with diethyl ether to yield 103.3 g (72%) of **1**; pure according to TLC. It was recrystallized from ethanol; m.p. 128–129°C (ref. 11, 124°C); $[\alpha]_D^{25}$ −229° (*c* 1.0, chloroform/ethanol 9 : 1). Calcd. for $C_{24}H_{29}NO_5$ (411.48): C 70.04, H 7.10, N 3.40; found: C 70.3, H 7.2, N 3.4. MS: M^+ 411. Pure according to HPLC (*k'* 1.24, methanol/water 60 : 40, 250 nm, 1 ml/min). ^{1}H NMR: δ 1.2 (t, 3H, $OCH_2C\underline{H}_3$), 2.4 (s, 3H, NCH_3), 3.6 (s, 3H, 6-OCH_3), 3.8 (s, 3H, 3-OCH_3), 4.1 (q, 2H, OCH_2), 4.6 (d, 1H, 5-βH), 5.6 (d, 1H, 17-H), 5.9 (q, 1H, 18-H), 6.6 (q, 2H, ArH).
Crystallization as hydrochloride from ethanol/ethyl acetate: m.p. 245°C (dec.), (ref. 11, 258°C), $[\alpha]_D^{25}$ −215° (*c* 1.0, water).

Ethyl 6,14-endo-*etheno-6,7,8,14-tetrahydrothebaine-7β-carboxylate* (**2**)

The filtrate of **1** was concentrated *in vacuo* to a smaller volume and set aside at room temperature until the 7β-adduct **2** crystallized. The shape of the crystals of **2** was different from that of **1** and the former was more soluble in diethyl ether. Using this difference, 5.6 g (4%) of pure **2** was obtained from 109 g of thebaine. It was recrystallized from ethanol; m.p. 110–111°C (ref. 11, 106–108°C); $[\alpha]_D^{25}$ −218° (*c* 1.0, chloroform/ethanol 9 : 1). Calcd. for $C_{24}H_{29}NO_5$ (411.48): C 70.04, H 7.10, N 3.40; found: C 70.0, H 7.0, N 3.6. MS: M^+ 411. ^{1}H NMR: δ 1.3 (t, 3H, $OCH_2C\underline{H}_3$), 2.4 (s, 3H, NCH_3), 3.5 (s, 3H, 6-OCH_3), 3.8 (s, 3H, 3-OCH_3), 4.2 (q, 2H, OCH_2), 5.2 (d, 1H, 5-βH), 5.5 (d, 1H, 17-H), 6.0 (q, 1H, 18-H), 6.6 (q, 2H, ArH). HPLC (*k'* 1.44, methanol/water 60 : 40, 250 nm, 1 ml/min) showed the product to be free from isomer **1**.

Ethyl 6,14-endo-*etheno-7,8-dihydromorphine-7β-carboxylate* (**3**)

A freshly prepared saturated solution of hydrogen bromide in glacial acetic acid (30 ml) was added to 4.1 g (10 mmol) of **2**. The mixture was set aside in the dark at room temperature for 90 h. The solvent was then evaporated *in vacuo* with the addition of some ethanol and the residue dissolved in 150 ml of a mixture of ethanol and water (1 : 1). Bromide ions were exchanged against chloride ions using 40 ml of anion exchanger AG 1-X8 [Cl^--form, 50–100 mesh, prewashed with 100 ml of a mixture of ethanol and water (1 : 1)] over 2 h. The mixture was filtered, the filtrate evaporated *in vacuo* and the residue boiled under reflux for 3 h with hydrogen chloride in ethanol. The solvent was then evaporated *in vacuo*. The residue was first liberated as a base in the usual manner and then treated with ethanolic hydrogen chloride to give 4.2 g (100%) of **3**·HCl; pure according to TLC. It crystallized readily from 2-propanol/ethyl acetate; m.p. 220°C (dec.); $[\alpha]_{546}^{25}$ −187° (*c* 1.0, water). Calcd. for $C_{22}H_{25}NO_5 \cdot HCl \cdot H_2O$ (437.91): C 60.34, H 6.45, N 3.20; found: C 60.4, H 6.4, N 3.1.

Ethyl 6,14-endo-*etheno-7,8-dihydromorphine-7α-carboxylate* (**4**)

a. With saturated hydrogen bromide in glacial acetic acid. The procedure as described for **3** was followed. From 9.5 g (23 mmol) of **1** 6.3 g of **4** (71%) was obtained. It was recrystallized from ethanol/water: m.p. 208–210°C (ref. 15, 194°C); $[\alpha]_D^{25}$ −205° (*c* 1.1, ethanol). Calcd. for $C_{22}H_{25}NO_5 \cdot 0.3H_2O$ (388.83): C 67.95, H 6.64, N 3.60; found: C 68.1, H 6.7, N 3.9. MS: M^+ 383 (no trace of OCH_3 = 383 + 14). ^{1}H NMR: δ 1.1 (t, 3H, $OCH_2C\underline{H}_3$), 2.4 (s, 3H, NCH_3), 4.1 (q, 2H, OCH_2), 4.4 (d, 1H, 5-βH), 5.4 (d, 1H, 17-H), 5.8 (q, 1H, 18-H), 6.5 (q, 2H, ArH).

b. With boron tribromide. A solution of 4.1 g (10 mmol) of **1** in 25 ml of anhydrous chloroform was added dropwise over about 4 min to a stirred solution of 175 ml of chloroform containing

[18] *G. Losse* and *H. Jeschkeit*, Chem. Ber. **90**, 1275 (1957).

16.5 g (70 mmol) of boron tribromide. The reaction mixture was stirred for 15 min and then poured into a mixture of 100 g of ice and 25 ml of concentrated ammonia. After stirring for a further 30 min, the reaction mixture was extracted with chloroform and worked up in the usual way. TLC, IR and mixed melting point showed a compound identical to that prepared above, **4**(a). A second portion of **4** was recovered from the hydrolyzed product in the aqueous layer by esterification. Compound **4** was crystallized as the HCl salt from hydrogen chloride in ethanol; m.p. 300°C (dec.); $[\alpha]_D^{25}$ −191° (*c* 1.0, water). Calcd. for $C_{22}H_{25}NO_5 \cdot HCl$ (418.90): C 62.93, H 6.24, N 3.34; found: C 62.9, H 6.4, N 3.4. MS: M^+ 383 (no trace of OCH_3).

c. Via esterification of **5**. Esterification of **5** was accomplished using ethanolic hydrogen chloride. Work up in the usual manner afforded, after recrystallization from ethanol/water, the same product as described under **4**(a). M.p. 204–207°C (a mixed melting point determination showed no depression). MS: M^+ 383. IR identical with **4**(a).

6,14-endo-*Etheno-7,8-dihydromorphine-7α-carboxylic acid* (**5**)

Compound **1** (5 g, 12.2 mmol) was heated at 90°C for 8 h in 50 ml of concentrated hydrogen bromide (47–49%). The reaction mixture was diluted with 100 ml of water and the solvent evaporated *in vacuo*. This procedure was repeated with 50 ml of water and twice with 25 ml of water, respectively. Finally, the residue was taken up in 10 ml of warm water and left at room temperature. The crystals were collected and washed with cold water, yielding 3.2 g (60%) of **5**·HBr; m.p. 276°C (dec.); $[\alpha]_D^{25}$ −160° (*c* 1.0, water). Calcd. for $C_{20}H_{21}NO_5 \cdot HBr$ (436.30): C 55.05, H 5.08, N 3.21; found: C 55.2, H 5.0, N 3.3. MS: M^+ 355. ^{1}H NMR (D_2O): δ 3.0 (s, 3H, NCH_3), 5.5 (d, 1H, 17-H), 5.8 (d, 1H, 18-H), 6.7 (s, 2H, ArH).

6,14-endo-*Etheno-6,7,8,14-tetrahydrothebaine-7α-carboxylic acid* (**6**)

Compound **2** (49.3 g, 120 mmol) was boiled under reflux for 7 h in a mixture of 70 ml of peroxide-free dioxane, 60 ml of water and 120 ml of 2 N sodium hydroxide. After standing overnight at room temperature, the reaction mixture was acidified with hydrogen chloride to pH 3 and evaporated *in vacuo* to dryness. The residue was taken up in 500 ml of water and unreacted **2** was removed by extraction at pH 8 with 50, 40 and 30 ml of chloroform, respectively. The aqueous layer was acidified to pH 2 with 2 N hydrogen chloride and evaporated *in vacuo* to dryness. The residue was recrystallized twice from water, yielding 41.7 g (82%) of **6**·HCl; m.p. 259°C (dec.); $[\alpha]_D^{25}$ −214° (*c* 1.5, water). Calcd. for $C_{22}H_{25}NO_5 \cdot HCl$ (419.90): C 62.93, H 6.24, N 3.34; found: C 62.7, H 6.2, N 3.4. ^{1}H NMR (DMSO): δ 2.9 (s, 3H, NCH_3), 3.5 (s, 3H, 6-OCH_3), 3.7 (s, 3H, 3-OCH_3), 4.9 (s, 1H, 5-βH), 5.5 (d, 1H, 17-H), 5.7 (d, 1H, 18-H), 6.7 (q, 2H, ArH). MS: M^+ 383. (Ref. 11, m.p. of **6**·HCl·H_2O 246–247°C.)

N-*(6,14*-endo-*Etheno-6,7,8,14-tetrahydrothebaine-7α-carbonyl)*-L-*leucine ethyl ester* (**7**)

A mixture of 7.25 g (17.25 mmol) of **6**, 150 ml of anhydrous 1,2-dichloroethane and 16 ml (175 mmol) of oxalyl chloride was boiled under reflux for 30 min. After cooling, the solvent was carefully evaporated *in vacuo* to dryness. Drying was twice repeated with the addition of some dichloromethane (ethanol-free) and finally at 0.1 mm Hg. The residue was dissolved in 100 ml of dichloromethane and the solution added over 60–70 min to a stirred mixture of 3.0 g (15 mmol) of L-leucine ethyl ester hydrochloride in 60 ml of dichloromethane and 6 ml (52.5 mmol) of NMM. After 45 min, 50 ml of dichloromethane and 50 ml of water were added and the aqueous layer adjusted to pH 7.5–8.0. The organic layer was separated and the aqueous layer extracted with 50 ml of dichloromethane. The combined organic layers were washed with 50 ml of hydrogen chloride in water (pH ~ 3) and 25 ml of water, respectively, dried over magnesium sulfate and evaporated *in vacuo*. The residue was taken up in hydrochloric ethanol and evaporated to dryness. The residue was crystallized from the mixture of 10 ml of ethanol and 20 ml of petroleum ether to yield 7.7 g (92%) of **7**·HCl which was pure, according to TLC and fluorescamine reaction; m.p. 162–169°C (after recrystallization); $[\alpha]_D^{25}$ −181° (*c* 1.4, ethanol). Calcd. for $C_{30}H_{40}N_2O_6 \cdot HCl$ (561.10): C 64.21, H 7.37, N 4.99; found: C 64.2, H 7.3, N 5.0. MS: M^+ 524. ^{1}H NMR: δ 0.8 (s, 3H, $CH_2C\underline{H}_3$), 0.9 (s, 3H, $CHC\underline{H}_3$), 1.2 (t, 3H, $OCH_2C\underline{H}_3$), 3.1 (s, 3H, NCH_3), 3.6 (s, 3H, 6-OCH_3), 3.8 (s, 3H, 3-OCH_3), 4.1 (q, 2H, $OC\underline{H}_2CH_3$), 4.7 (d, 1H, 5,βH), 5.5 (d, 1H, 17-H), 6.0 (d, 1H, 18-H), 6.6 (q, 2H, ArH). Pure according to HPLC.

N-*(6,14*-endo-*Etheno-6,7,8,14-tetrahydrothebaine-7α-carbonyl)*-L-*phenylalanyl*-L-*leucine ethyl ester* (**8**)

The same procedure as described for **7** was followed for the preparation of **8**, with a yield of 85%. The *N*-protecting group was removed in the usual way with trifluoroacetic acid and dichloromethane (1 : 1). **8**·HCl was recrystallized from a mixture of ethanol and ethyl acetate; m.p. 222°C (dec.); $[\alpha]_D^{25}$ −180° (*c* 1.3, water). Calcd. for $C_{39}H_{49}N_3O_7 \cdot HCl$ (780.27): C 66.13, H 7.12, N 5.93; found: C 66.3, H 7.1, N 5.8.

N-*(6,14*-endo-*Etheno-6,7,8,14-tetrahydrothebaine-7α-carbonyl)glycyl*-L-*phenylalanyl*-L-*leucine ethyl ester* (**9**)

The same procedure as described for **7** was followed for the preparation of **9**·HCl, which was recrystallized from ethanol; yield 82%; m.p. 230–232°C; $[\alpha]_D^{25}$ −144° (*c* 1.0, ethanol). Calcd. for $C_{41}H_{52}N_4O_8 \cdot HCl \cdot \frac{1}{2}H_2O$ (774.33): C 63.59, H 7.03, N 7.24; found: C 63.8, H 7.3, N 7.4.

N-*(6,14*-endo-*Etheno-6,7,8,14-tetrahydrothebaine-7α-carbonyl)*-L-*phenylalanyl*-L-*leucinol* (**10**)

The hydrochloride of **8** (1 g, 1.4 mmol) was converted into the base in the usual way and taken up in 15 ml of anhydrous 2-propanol. To this solution, 500 mg (4.5 mmol) of anhydrous calcium chloride and 144 mg (3 mmol) of sodium tetrahydroborate were added. After two days, a further portion of 72 mg (1.5 mmol) of sodium tetrahydroborate was added. After 5 days, the reaction was complete according to TLC. To the reaction mixture, 25 ml of water was added. The solution was acidified to pH 2–3 with 2 N hydrogen chloride and stirred for 30 min. Extraction with chloroform at pH 8 and work up in the usual way yielded 820 mg (92%) of **10** which was recrystallized from ethyl acetate/petroleum ether (4°C); m.p. 97–99°C; $[\alpha]_D^{25}$ −195° (*c* 1.0, ethanol). Calcd. for $C_{37}H_{47}N_3O_6 \cdot \frac{1}{2}H_2O$ (639.78): C 69.57, H 7.57, N 6.58; found: C 69.6, H 7.4, N 6.4. MS: M^+ 629.

N-*(6,14*-endo-*Etheno-7,8-dihydromorphine-7α-carbonyl)*-L-*leucine ethyl ester* (**11**)

a. With hydrogen bromide in glacial acetic acid. To 5.61 g (10 mmol) of **7**·HCl, which was converted to the free base, 30 ml of a freshly prepared saturated solution of hydrogen bromide in glacial acetic acid was added. The mixture was set aside in the dark at room temperature for 115 h. The solvent was then evaporated *in vacuo* (35°C), the residue taken up in 10 ml of water and again evaporated *in vacuo* to dryness. The residue was dissolved in 200 ml of water. Bromide ions were exchanged for chloride ions using 80 ml of anion exchanger AG 1-X8 (Cl^--form, 50–100 mesh). After 1 h, the exchanger was filtered off and washed with 100 ml of ethanol. The filtrate was evaporated *in vacuo* to dryness and the residue esterified with 100 ml of hydrogen chloride in ethanol. The mixture was evaporated to dryness *in vacuo* to yield 4.5 g (84%) of **11**·HCl, which was pure according to TLC. It was purified by dissolving in a small volume of ethanol and precipitating in diethyl ether; m.p. 195°C (dec.); $[\alpha]_D^{25}$ −183° (*c* 1.0, water). ^{1}H NMR: no signals of OCH_3 at δ 3.6 and 3.8. Pure according to HPLC.

b. With boron tribromide. The conversion was carried out as described for **4**(*b*). ^{1}H NMR and IR spectra were identical with those of **11**(*a*).

N-*(6,14*-endo-*Etheno-7,8-dihydromorphine-7α-carbonyl)*-L-*phenylalanyl*-L-*leucine ethyl ester* (**12**)

From the hydrochloride of **8** we obtained **12**·HCl using the same procedure as described for **11** (yield 89%). It was recrystallized from ethanol; m.p. 215°C (dec.); $[\alpha]_D^{25}$ −154° (*c* 1.0, water). ^{1}H NMR: no signals of OCH_3 at δ 3.6 and 3.8. MS: M^+ 643. Pure according to HPLC.

N-*(6,14*-endo-*Etheno-7,8-dihydromorphine-7α-carbonyl)glycyl*-L-*phenylalanyl*-L-*leucine ethyl ester* (**13**)

Compound **13**·HCl was obtained from **9** using the same procedure as described for **11** (yield 73%). It was purified (TLC) by fractional

precipitation from ethanol and diethyl ether, m.p. 240°C (dec.); $[\alpha]_D^{25}$ −140° (*c* 1.0, water). MS: M^+ 700.

N-*(6,14*-endo-*Etheno-7,8-dihydromorphine-7α-carbonyl)*-L-*-phenylalanyl*-L-*leucinol* (**14**)

The hydrochloride of **12** (1.63 g, 2.4 mmol) was converted into the base and dissolved in 30 ml of anhydrous 2-propanol. To this solution, 1.5 g (15 mmol) of anhydrous calcium chloride and 1.14 g (30 mmol) of sodium tetrahydroborate were added. The conversion was complete (TLC) after 6 days at 35°C. Water (50 ml) was then added and the mixture acidified with 2 N hydrogen chloride to pH 2–3. Extraction with a mixture of chloroform and 2-propanol (2 : 1) at pH 8 and work up in the usual way, yielded 1.2 g (78%) of **14**, which was pure according to TLC. It was recrystallized from aqueous ethanol; m.p. 235°C (dec.); $[\alpha]_D^{25}$ −172° (*c* 0.3, 0.1 N hydrogen chloride). Calcd. for $C_{35}H_{43}N_3O_6$ (601.72): C 69.86, H 7.20, N 6.98; found: C 69.5, H 7.3, N 6.7. MS: M^+ 601. Pure according to HPLC.

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Trimethylacetic formic anhydride. Improved preparation and use as a highly efficient and selective N-formylating reagent

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Abstract. The title compound was prepared from trimethylacetyl chloride and sodium formate in the presence of 18-crown-6. The mixed anhydride proved to be a very useful reagent for the N-formylation of amines. Even amines which are unstable when deprotonated can be formylated.

Recently, we reported the synthesis of methyl 2-([^{13}C,^{15}N]-isocyano)propionate[1]. A key step in this synthesis is the introduction of the ^{13}C label in the methyl ester of *N*-([^{13}C]formyl)[^{15}N]alanine, the compound from which the isocyanide is prepared. Introduction occurs by reacting [^{15}N]alanine methyl ester with separately prepared acetic [^{13}C]formic anhydride. This reaction also affords the corresponding acetamide in 8% yield. Presumably this latter compound results from nucleophilic attack of the amine nitrogen on the acetyl moiety of the mixed anhydride and/or the acetic anhydride formed by disproportionation[2]. Suppression of this reaction should be possible by making the acetyl side of the mixed anhydride less susceptible to nucleophilic attack. This can be achieved by substituting the α-carbon atom of the acetyl part with groups having appropriate steric and/or electric properties. The most obvious compound having these features is trimethylacetic formic anhydride.
Although the existence of the latter compound has been suggested in earlier reports, *Schijf* and *Stevens*[3] were the first to unambiguously establish its presence using NMR spectroscopy. They obtained the mixed anhydride in 60% isolated yield by reacting trimethylacetyl chloride with sodium formate in THF at 0°C for 24 h. The distilled product contained 1–2 mol % of the corresponding symmetrical anhydrides. Closer investigation[4] revealed that the moderate yield is due to decomposition, both thermally, during the long reaction time, removal of the solvent and distillation of the anhydride, and chemically, by sodium formate and traces of carboxylic acids. The instability of the product obtained by the procedure of *Schijf* and *Stevens* was confirmed by *Harman* and *Hutchinson*[5], who failed to isolate the pure anhydride by distillation at room temperature and 18 mm Hg.
We wish to report an improvement on the literature procedure for the preparation of trimethylacetic formic anhydride. In our procedure the compound is formed in a fast reaction and no separation of solvent is necessary, resulting in effective suppression of the various decomposition paths open to the mixed anhydride.
The title compound can be prepared, without using a solvent, from trimethylacetyl chloride and 1.05 equivalent of sodium formate in the presence of 10 mol % 18-crown-6 at 0°C. The reaction is complete within 3 h and the product can be isolated directly from the reaction mixture by distillation into a cold trap at 0.01 mmHg and 0°C. Yields vary from 95 to 98%. According to ^{1}H NMR, the only contaminants are traces (less than 1%) of trimethylacetic acid,

[1] *E. J. Vlietstra, R. J. M. Nolte, J. W. Zwikker, W. Drenth* and *R. H. A. M. Janssen*, Recl. Trav. Chim. Pays-Bas **101**, 183 (1982).
[2] *W. Stevens* and *A. van Es*, Recl. Chim. Pays-Bas **83**, 863 (1964).
[3] *R. Schijf* and *W. Stevens*, Recl. Trav. Chim. Pays-Bas **85**, 627 (1966).
[4] *R. Schijf*, Ph.D. Thesis, State University of Leiden, The Netherlands, 1968.
[5] *A. D. Harmon* and *C. R. Hutchinson*, J. Org. Chem. **40**, 3474 (1975).