Peptides as Receptor Selectivity Modulators of Opiate Pharmacophores[1]

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In an effort to investigate whether "address" segments of endogenous opioid peptides, which are responsible for modulating receptor selectivity, also could modulate the selectivity of opioid alkaloid pharmacophores, we have synthesized analogues of leucine-enkephalin and dynorphin(1–8) in which the N-terminal dipeptide "message" sequence has been replaced by oxymorphone or naltrexone. A hydrazone group was employed as a linkage between the alkaloids and peptides. The binding data for μ, κ, and δ receptors indicate that peptide portions of the analogues can modulate the receptor selectivity of the attached alkaloid pharmacophores. The selectivity for different opioid receptor types depends on a balance between the affinities of the message and address components. In cases where these components have comparable receptor affinities, the address can significantly shift selectivity by increasing affinity to one receptor type while reducing affinity to other types. When the message component has high affinity for a particular receptor type, the modulatory role of the address is expressed mainly by reducing the affinity of the ligand for other opioid receptor types.

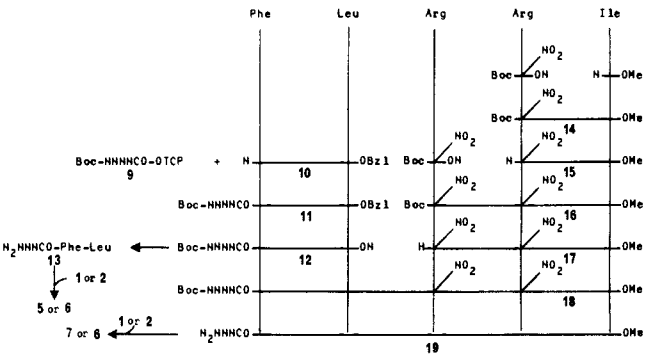
Since the isolation of the enkephalins,[3] numerous endogenous peptide analogues have been characterized.[4,5] Most of these opioid peptides contain an N-terminal tetrapeptide fragment identical with the enkephalins. Biological studies of these peptides, as well as numerous synthetic analogues, have led to the conclusion that the tetrapeptide sequence from Tyr[1] to Phe[4] is an important requirement for the activity. It has been proposed that the N-terminal tetrapeptide sequence of endogenous peptides carries the "message", which is responsible for mediating the opioid effect. The C-terminal segments of these peptides, which differ in length and physiochemical character, play an "address" role in conferring selectivity for different opioid receptor types.[6]

While there appears to be cross-reactivity of the opioid peptides and alkaloids with respect to μ, κ, and δ receptors, structure–activity relationship studies of opiates and related alkaloids and opioid peptides have yielded no firm conclusions regarding their common pharmacophoric elements. In this regard, it is probable that the mode of interaction with the same receptor is different for conformationally rigid alkaloids and flexible peptide molecules.[7] The report[8] that simple replacement of the tyramine moiety of tyrosine in enkephalin by an opiate structure affords an inactive ligand is consistent with this idea.

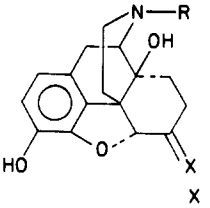
One explanation for the inability of opiates to replace tyrosine is the possibility that the N-terminal dipeptide moiety of the opioid peptides may be relevant to the morphine alkaloids, and that this element carries the opioid message. This endogenous peptide pharmacophore needs supporting elements, the address, which could enhance the binding between the opioid peptide and a particular receptor type. This model relegates the Phe[4] residue to the address segment of the opioid peptide.

In an effort to investigate this model, it was of interest to determine whether or not the presumed address elements of opioid peptides can modulate receptor selectivity of an alkaloid pharmacophore. In this paper, we present the synthesis of analogues in which oxymorphone **1** and naltrexone **2** are connected through a hydrazone linkage to peptides related to an address portion of enkephalin and dynorphin(1–8). Receptor binding of the alkaloid semicarbazones (**3**, **4**) and the corresponding hybrid alkaloid–peptides (**5–8**) suggest that the presumed peptide address

Scheme I



is capable of modulating the receptor selectivity of the alkaloid–peptide hybrid in a predictable fashion.



R	X
1. CH_3	O
2. $CH_2CH(CH_2)_2$	O
3. CH_3	$NNHCONH_2$
4. $CH_2CH(CH_2)_2$	$NNHCONH_2$
5. CH_3	NNHCO-Phe-Leu
6. $CH_2CH(CH_2)_2$	NNHCO-Phe-Leu
7. CH_3	NNHCO-Phe-Leu-Arg-Arg-IleOMe
8. $CH_2CH(CH_2)_2$	NNHCO-Phe-Leu-Arg-Arg-IleOMe

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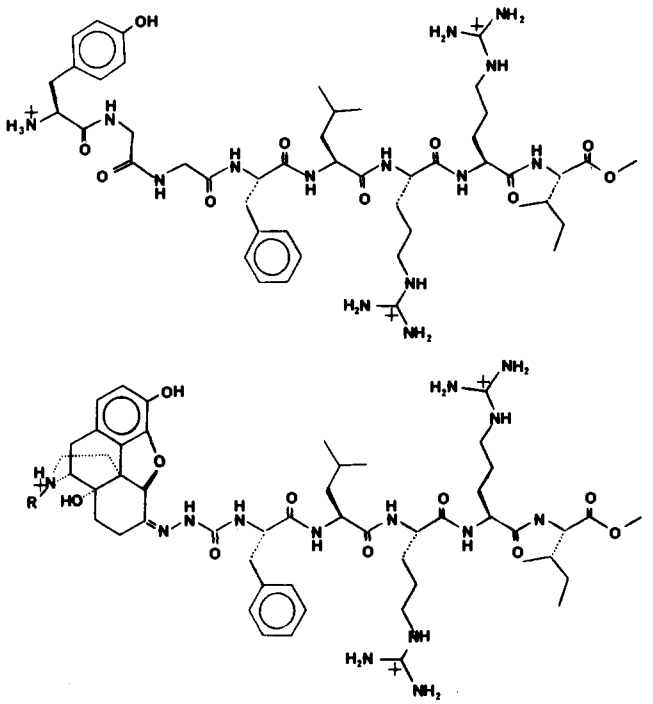

Figure 1. Structural comparison of dynorphin(1–8)OMe (A) and the alkaloid–dynorphin "address" hybrid (B) (R = methyl or cyclopropylmethyl).

Design Rationale and Chemistry

The rationale for the design of the alkaloid–peptide hybrid components was based upon the known δ and κ selectivity of leucine-enkephalin and dynorphin(1–8), respectively. Accordingly, we speculated that the presumed address sequences, Phe-Leu and Phe-Leu-Arg-Arg-IleOMe, in these peptides might confer δ and κ selectivity to the hybrid molecules **5–8** (Figure 1). The latter address sequence was introduced in the form of the methyl ester because its was reported[6] that it possesses greater κ activity than dynorphin(1–8). Also, the opiate semicarbazones **3** and **4** were envisaged to correspond to the N-terminal dipeptide amide sequence in the opioid peptides. The semicarbazone group was a convenient moiety through which the opiate could be linked to the peptides in this study.

The synthetic routes to the hybrid alkaloid–peptides are outlined in Scheme I. The peptide address sequences were synthesized by the solution method using DCC in the presence of HOBt. The nitro group was used in the protection of the guanidine function of the arginine residue. The benzyl and methyl esters were employed in protecting the C-terminus of leucine and isoleucine, respectively. Boc-azaglycine was coupled to protected peptides via its active ester **9**. After removal of benzyl, nitro, or Boc protecting groups, the peptides **12** or **19** were condensed with the alkaloids. The final hybrid peptides **5–8** were purified by gel filtration on Sephadex-LH20 in methanol. The semicarbazones **3** and **4** were synthesized by reaction of the corresponding ketones **1** and **2** with semicarbazide.

Results and Discussion

In accordance with the model, the different peptide addresses attached to the oxymorphone pharmacophore changed the receptor selectivity of the ligands (Table I). Thus, combination with the enkephalin address (**5**) increased the selectivity for δ receptors, and attachment of the dynorphin(1–8) address (**7**) increased the selectivity for κ receptors when compared to oxymorphone **1** and its semicarbazone **3**. In the case of compound **5** with the enkephalin address, the changes in selectivity were mainly the result of enhancement of affinity for δ opioid receptors. Elongation of the peptide component with Arg-Arg-IleOMe resulted in **7** with a dynorphin(1–8) address, and its observed change in receptor selectivity appears to be a consequence of a marked increase in κ receptor affinity with a concomitant decrease in δ receptor affinity. It is noteworthy that all members of the oxymorphone series (**1**, **3**, **5**, **7**) have nearly identical affinities at μ receptors. This may mean that subsites outside the μ binding site do not recognize any elements of the peptide chain, when it is attached to the oxymorphone pharmacophore.

Table I. Binding of Alkaloid–Peptide Hybrids to Opioid Receptor Types in Guinea Pig Membranes[a]

compound	binding: K_i,[b] nM		
	μ	κ	δ
1	15 ± 1	725 ± 154	145 ± 17
3	14.5 ± 0.97	179 ± 22	69 ± 2.7
5	14.8 ± 1.8	96 ± 13	6.5 ± 0.18
7	15.5 ± 0.94	9.8 ± 0.21	24 ± 0.84
2	0.37 ± 0.03	4.8 ± 1.0	9.4 ± 1.0
4	0.48 ± 0.02	2.6 ± 0.24	3.7 ± 0.30
6	0.76 ± 0.08	2.8 ± 0.20	3.1 ± 0.10
8	2.3 ± 0.36	2.2 ± 0.41	5.7 ± 0.60
leucine-enkephalin	380 ± 45	>10000	6.0 ± 0.5
dynorphin(1–8)	340 ± 25	40 ± 14	50 ± 18

[a] Prepared by the method described by Tam: S. W. Tam, *Eur. J. Pharmacol.* **1985**, *109*, 33. [b] Values obtained from at least three replicate determinations. The following radioligands were employed: 0.5 nM of [^{3}H]naloxone (μ binding); 1 nM of (−)-[^{3}H]-ethylketazocine in the presence of DADLE (500 nM) and sufentanil (20 nM) (κ binding); 1 nM of [^{3}H]DADLE in presence of sufentanil (4 nM) (δ binding). All binding were performed in 50 mM Tris buffer, pH 7.4, containing 100 mM NaCl and bacitracin (50 μg/mL).

In the case of the naltrexone series, the ability of the address portion of the molecule to modulate selectivity appears to be overshadowed by the greater affinity of this pharmacophore relative to the corresponding oxymorphone analogues. Thus, the changes in affinity afforded by the joining of the peptides to naltrexone were considerably less than those observed in the oxymorphone series. The rather marginal selectivity changes that occurred upon such modification arose from binding differences at μ sites. Accordingly, dynorphin analogue **8** has greater selectivity at κ sites compared to compounds **4** and **6** because **8** possesses lower affinity than the latter at μ receptors. In this regard, it is of interest that the increase in κ selectivity of **8** occurred without a significant change in its affinity for κ sites. These data draw attention to the fact that selectivity changes upon molecular modification can arise by two basically different mechanisms: (1) by an affinity increase of a ligand for a particular binding site (e.g., oxymorphone series), and/or (2) by decreasing the affinity of the ligand at other sites. It can be noted that the affinity of naltrexone **2** and its semicarbazone **4** for opioid receptors is considerably greater than the corresponding oxymorphone compounds (**1**, **3**). The fact that the address components are expressed to a greater degree in the oxymorphone series suggests that the modulation of selectivity effected by the address segment may depend on a balance between the affinities of the message and of the address components. In cases where the message and address components have comparable receptor affinities, one would expect the address component would significantly modify selectivity by increasing affinity to one receptor type over other receptor types. On the other hand, in the cases where the message component has substantially higher affinity for the receptor, the modulatory role of the address may be expressed to a minimal extent. These results are

consistent with the message–address concept and suggest an approach to modifying the selectivity of opiates at opioid receptors.

Experimental Section

Melting points were determined with a Mel-Temp capillary apparatus and are uncorrected. Optical rotations were taken with a 10-cm cell on Autopol III. TLC plates, silica gel GF, 0.25-mm thick, were obtained from Analtech, Inc. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. Amino acids analyses were determined with a Beckman 118BL amino analyzer. All reagents and solvents were reagent grade and were used without subsequent purification. Abbreviations employed are as follows: DADLE, [D-Ala^2,D-Leu^5]enkephalin; TEA, triethylamine; DMF, *N*,*N*-dimethylformamide; DCC, *N*,*N*′-dicyclohexylcarbodimide; HOBt, *N*-hydroxybenzotriazole.

Oxymorphone Semicarbazone Hydrochloride (3). Oxymorphone (30 mg, 0.1 mmol) and semicarbazide hydrochloride (13 mg, 0.11 mmol) were refluxed for 2 h in methanol (5 mL). After concentation of the solution to 1 mL, the mixture was chromatographed on Sephadex LH-20 with methanol as eluent to afford 40 mg (95%) of product: mp 261 °C dec; $[\alpha]^{23}_D$ −320° (*c* 1, DMF); R_f 0.04 (CH_2Cl_2/MeOH/NH_4OH, 95:5:0.5). Anal. ($C_{18}H_{22}N_4O_4$·HCl·1.5H_2O) C, H; N: calcd, 13.28; found, 12.72.

Naltrexone Semicarbazone (4). Naltrexone hydrochloride (37.7 mg, 0.1 mmol) and semicarbazide hydrochloride (13 mg, 0.11 mmol) were suspended in methanol (5 mL), and TEA (0.03 mL) was added. After 2 h of stirring under reflux, a 5% Na_2CO_3 (20 mL) was added to the solution, and aqueous solution was extracted with ethyl acetate (three times). The ethyl acetate fraction was dried ($MgSO_4$) and evaporated, and the residue was washed with ethyl ether. Yield 33.6 mg (84%); mp 170–173.5 °C; $[\alpha]^{23}_D$ − 354° (*c* 1, DMF); R_f 0.05 (CH_2Cl_2/MeOH/NH_4OH, 95:5:0.5); R_f 0.51 ($CHCl_3$/MeOH, 10:2). Anal. ($C_{21}H_{26}N_4O_4$·H_2O) C, H, N.

***N*-[[*N*^{2}-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]-phenylalanylleucine Benzyl Ester (11).** A cold (−10 °C) $CHCl_3$ (5 mL) solution of 2,4,5-trichlorophenyl chloroformate (520 mg, 2 mmol) was added with stirring to a cold $CHCl_3$ (10 mL) solution containing (*tert*-butoxycarbonyl)hydrazine (Boc-$NHNH_2$) (264 mg, 2 mmol) and TEA (404 mg, 4 mmol). After stirring overnight at 23 °C to assure complete conversion to the active ester **9**, Phe-LeuOBzl·HCl (**10**; 810 mg, 2 mmol) dissolved in DMF (2 mL) was added, followed in 1 h by HOBt (270 mg, 2 mmol). The reaction mixture was stirred for 15 h at 23 °C and extracted with ethyl acetate (50 mL), and the organic phase was washed (three times) with aqueous 1 N Na_2CO_3, 10% citric acid, and water. The dried ($MgSO_4$) ethyl acetate was removed in vacuo and ethyl ether (20 mL) was added to the oily residue to afford 856 mg (81%) of **11**: mp 156–159 °C; $[\alpha]^{23}_D$ −10.6° (*c* 1, DMF), R_f 0.90 ($CHCl_3$/MeOH, 2:1). Anal. ($C_{28}H_{38}N_4O_6$·1.5H_2O) C, H, N.

***N*-[[*N*^{2}-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]-phenylalanylleucine (12).** Compound **11** (831 mg, 1.5 mmol) was dissolved in methanol (50 mL) containing 10% of DMF and stirred for 4 h with 10% Pd/C (50 mg) under H_2 at atmospheric pressure. After filtration, the solution was concentrated to 5 mL, 1 N Na_2CO_3 (20 mL) was added, and the aqueous solution was extracted (three times) with ethyl acetate. The aqueous solution then was acidified with 10% citric acid and extracted (three times) with ethyl acetate. After drying of the extracts ($MgSO_4$) and concentration to 10 mL, petroleum ether (50 mL) was added to afford **12**, which was isolated by filtration: yield 600 mg (92%); mp 97–99 °C; R_f 0.55 (MeOH/$CHCl_3$, 1:2); $[\alpha]^{23}_D$ −12.5° (*c* 1, DMF). Anal. ($C_{21}H_{32}N_4O_6$·1.5H_2O) C, H; N: calcd, 12.09; found, 11.30.

Oxymorphone *N*-(Hydrazinocarbonyl)phenylalanylleucine Hydrochloride Derivative (5). Compound **12** (93 mg, 0.2 mmol) was dissolved in 4 N HCl in ethyl acetate (5 mL). After the reaction mixture was allowed to stand for 15 min, the product, H_2NNHCO-Phe-Leu·HCl (**13**·HCl) was filtered and washed with ethyl ether. The yield of chromatographically pure **13**·HCl (R_f 0.32, *n*-BuOH/AcOH/H_2O, 2:1:1) was 72 mg (97%). This intermediate (37 mg, 0.1 mmol) and oxymorphone **1** (30 mg, 0.1 mmol) were dissolved in methanol (10 mL), and the solution was refluxed for 1 h. After concentration of the reaction mixture to 0.5 mL, the product **5** was purified chromatographically on Sephadex LH-20 in methanol. The major fraction with R_f 0.63 (*n*-BuOH/AcOH/H_2O/, 3:1:1) was collected, concentrated to 5 mL, and precipitated by addition of ethyl acetate (30 mL). Yield 45 mg (69%); mp 252 °C dec; $[\alpha]^{23}_D$ −360° (*c* 1, DMF). Anal. ($C_{33}H_{41}N_5O_7$·HCl·2.5H_2O) C, H, N.

Naltrexone *N*-(Hydrazinocarbonyl)phenylalanylleucine Hydrochloride Derivative (6). Intermediate **13** (37 mg, 0.1 mmol) and naltrexone hydrochloride (37.7, 0.1 mmol) were dissolved in methanol (10 mL), and the solution was refluxed for 1 h. After concentration of reaction mixture to 0.5 mL, the product was purified by chromatography on Sephadex LH-20 in methanol. The major fractions with R_f 0.80 (*n*-BuOH/AcOH/H_2O, 3:1:1) were collected, concentrated to 5 mL, and precipitated by addition of ethyl ether (30 mL) to afford 56 mg (80%) of **6**: mp 261 °C dec; $[\alpha]^{23}_D$ −254° (*c* 0.7, DMF). Anal. ($C_{36}H_{45}N_5O_7$·HCl·2H_2O) C, H, N.

(*tert*-Butoxycarbonyl)-*N*^{G}-nitroarginylisoleucyl Methyl Ester (14). DCC (2.1 g, 10 mmol) was added to a stirred ice-cold DMF (30 mL) solution containing IleOMe·HCl (1.85 g, 10 mmol), TEA (1.4 mL), Boc-Arg(NO_2) (3.19 g, 10 mmol), and HOBt (2.7 g, 20 mmol). The mixture was stirred for 1 h in an ice bath and then for 15 h at 23 °C. Ethyl acetate (100 mL) was added to this mixture, and the precipitated dicyclohexylurea (DCU) was filtered and washed with ethyl acetate. The filtrate was washed successively with 10% $NaHCO_3$, water, 10% citric acid, and water. After drying ($MgSO_4$), the ethyl acetate was evaporated in vacuo. The residual oil was triturated with ethyl ether to afford **14** as a solid, which was recrystallized from 2-propanol. Yield 4.42 g (95%); R_f 0.75 (*n*-BuOH/AcOH/H_2O, 3:1:1); mp 154–156 °C; $[\alpha]^{23}_D$ −6.5° (*c* 1, DMF). Anal. ($C_{18}H_{34}N_6O_7$) C, H, N.

(*tert*-Butoxycarbonyl)-*N*^{G}-nitroarginyl-*N*^{G}-nitroarginylisoleucine Methyl Ester (16). Dissolution of intermediate **14** (3.71 g, 8 mmol) in 4 N HCl in ethyl acetate afforded compound **15**·HCl as a precipitate upon standing. After addition of ethyl ether, the mixture was decanted, and the solid was washed with ethyl ether and dried in vacuo. This chromatographically homogeneous (R_f 0.60, *n*-BuOH/AcOH/H_2O) intermediate was dissolved in DMF (30 mL) and TEA (1.4 mL) was added. After stirring 0.5 h at 23 °C, the reaction mixture was cooled in an ice bath, and Boc-Arg(NO_2) (2.55 g, 8 mmol) and HOBt (2.7 g, 20 mmol) were added. After an additional 10 min, DCC (168 g, 8 mmol) was added, and the mixture was stirred for 2 h at 0 °C and then for 15 h at 23 °C. The precipitated DCU was removed by filtration and the filtrate was diluted with 10% $NaHCO_3$ (100 mL). The precipitated solid was filtered and washed with 10% $NaHCO_3$, water, 10% citric acid, and again with water. Crystallization from 2-propanol afforded 3.96 g (79%) of **16**: R_f 0.68 (*n*-BuOH/AcOH/H_2O, 3:1:1); mp 101–103 °C; $[\alpha]^{23}_D$ −9.3° (*c* 1, DMF). Anal. ($C_{24}H_{45}N_{11}O_{10}$·C_3H_7OH·H_2O) C, N; H: calcd, 7.65; found, 7.05.

***N*^{G}-Nitroarginyl-*N*^{G}-nitroarginylisoleucine Methyl Ester Hydrochloride (17).** Compound **16** (3.17 g, 5 mmol) was dissolved in 4 N HCl in acetic acid (10 mL). After the solution was stirred for 0.5 h, a mixture (30 mL) of ethyl acetate and ethyl ether (1:1) was added. The precipitated crude product, **17**·HCl, was removed by filtration and was crystallized from a mixture 2-propanol/ethanol. Yield 2.69 g (89%), mp 200–201 °C; $[\alpha]^{23}_D$ −10.2° (*c* 0.3, DMF); R_f 0.50 (*n*-BuOH/AcOH/H_2O, 3:1:1). Anal. ($C_{19}H_{37}N_{11}O_8$·HCl·H_2O) C, H, N.

***N*-[[*N*^{2}-(*tert*-Butoxycarbonyl)hydrazino]carbonyl]-phenylalanylleucyl-*N*^{G}-nitroarginyl-*N*^{G}-nitroarginylisoleucine Methyl Ester (18).** Intermediate **17**·HCl (114 mg, 0.2 mmol) was dissolved in DMF (5 mL), and 0.3 mL of TEA was added. After stirring at 23 °C for 0.5 h, the reaction mixture was cooled to 0 °C, and compound **12** (92.6 mg, 0.2 mmol) and HOBt (54 mg, 0.4 mmol) were added. After 10 min this was followed by the addition of DCC (42 mg, 0.2 mmol) and stirring for 2 h at 0 °C and 15 h at 23 °C. Ethyl acetate (5 mL) then was added, and the precipitated DCU was removed by filtration. Ethyl ether (50 mL) was added to the filtrate, and the precipitate was collected. After crystallization from hot ethyl acetate/ethyl ether, 170 mg (88%) of **18** was obtained: R_f 0.32 (*n*-BuOH/AcOH/H_2O, 3:1:1); mp 252–253 °C; $[\alpha]^{23}_D$ −13.0° (*c* 1, DMF). Anal. ($C_{40}H_{67}N_{15}O_{13}$) C, H; N: calcd, 25.59; found 24.84.

Oxymorphone *N*-(Hydrazinocarbonyl)phenylalanylleucylarginylarginylisoleucine Methyl Ester Hydrochloride Derivative (7). Intermediate **18** (145 mg, 0.15 mmol) was dis-

solved in 4 N HCl in acetic acid (10 mL) containing 0.1% of anisole. After the mixture was allowed to stand for 10 min, methanol (75 mL) and 10% Pd/C (20 mg) were added. The mixture was hydrogenated for 12 h at atmospheric pressure, and the mixture was filtered free of catalyst. The filtrate was concentrated to 30 mL and ethyl ether (100 mL) was added. The precipitated solid was decantated and washed with ethyl ether and was dried over NaOH. The yield of this chromatographically purified (Sephadex LH-20 in methanol) intermediate **19** (R_f 0.1; *n*-BuOH/ACOH/H_2O, 3:1:1) was 111.2 mg (86%). Oxymorphone (20 mg, 0.075 mmol) was added to a methanol solution (5 mL) of the deprotected peptide **19** and the mixture was heated under reflux for 4 h. The solution was then concentrated to 1 mL and chromatographed on Sephadex LH-20 in methanol. The fractions containing the pure product **7** (R_f 0.18, *n*-BuOH/AcOH/H_2O, 3:1:1) were collected and evaporated to dryness. Yield 71 mg (78%); mp 252 °C dec; $[\alpha]^{23}_D$ −93.1° (*c* 1, DMF). Amino acids analysis: Arg:Ile:Leu:Phe; 1.91:1.02:1.00:0.98. Anal. ($C_{52}H_{78}N_{14}O_{10}\cdot 3HCl\cdot 3H_2O$) C, H, N.

Naltrexone *N*-(Hydrazinocarbonyl)phenylalanylleucylarginylarginylisoleucine Methyl Ester Hydrochloride Derivative (8). Naltrexone hydrochloride (28.3 mg, 0.075 mmol) was dissolved in 5 mL of a methanol solution of compound **19** (0.075 mmol), and the mixture was heated under reflux for 4 h. After concentration of solution to 1 mL, the product was chromatographed on Sephadex LH-20 in methanol. The TLC-pure fraction with R_f 0.22 (*n*-BuOH/AcOH/H_2O, 3:1:1) was collected and the solvent removed. Yield 72 mg (75%); mp 260 °C dec; $[\alpha]^{23}_D$ −110.5° (*c* 1, DMF). Amino acid analysis: Arg:Ile:Leu:Phe, 1.90:0.99:1.00:1.01. Anal. ($C_{55}H_{82}N_{14}O_{10}\cdot 3HCl\cdot 4H_2O$) C, H, N.

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New Cyanomorpholinyl Byproduct of Doxorubicin Reductive Alkylation

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Previously we reported that reductive alkylation of doxorubicin with 2,2′-oxybis[acetaldehyde] and $NaBH_3CN$ to form the 4″-morpholinyl derivative also gave the intensely potent 3″-cyano-4″-morpholinyl as a byproduct, by addition of CN^- to an iminium intermediate in place of hydride. We now find that sugar 4′-OH is a third nucleophile that can add to the iminium intermediate in this reaction. Bridging of the 4′-OH to the morpholine ring at C.5″ formed a novel byproduct with an oxazolidino ring fused to the sugar and morpholine. The new product was minor at neutral pH but predominant at an acidic pH. When tested against tumors in mice it was 4–6 times more potent than doxorubicin. Hence, in comparison with the 3″-cyano-4″-morpholinyl, potency was reduced up to 100-fold by the O bridge. Analytical HPLC showed the presence of three of the four possible diastereoisomers, and two were isolated. The diastereoisomers appeared to differ in stability. In vitro tests suggested that biological potency varied inversely with stability.

The Borch reductive alkylation of daunorubicin and doxorubicin with 2,2′-oxybis[acetaldehyde] (**7**) and $NaBH_3CN$ has given cyanomorpholino byproducts[1] (**2** and **4**, respectively) in addition to the expected morpholino derivatives[1,2] (**1** and **3**) that incorporate the amino N of the daunosamine moiety. The nonbasic cyanomorpholino derivative **4** of doxorubicin showed 100- to 1000-fold increases in antitumor potency, absence of cardiotoxicity, and activity against tumors that are resistant to doxorubicin.[1,3-7] This constitutes one of the strongest leads in the field of anthracycline drug development and analogue synthesis. We now report isolation of the O-bridged oxazolidino compounds **5** and **6** as additional active byproducts from the reductive alkylation.

Synthesis

Generally, the reaction was carried out in aqueous acetonitrile with a neutral solution of **7** in 15-fold excess, which was mixed with 1 molar equiv of $NaBH_3CN$ ($2H^-$ required for the morpholine or 1 for the cyanomorpholine), and finally with doxorubicin hydrochloride. Attempts to improve the yield of **4** through systematic changes in reaction time, stoichiometry, order of mixing, or addition of NaCN were consistently unsuccessful. The usual yields (HPLC analysis) of **3** (42%), **4** (23%), and accompanying 13-dihydro derivatives (21%, 10%) were virtually unaffected. Similarly, small changes in pH made little difference. When, however, acidity was significantly increased by adjusting the dialdehyde solution to pH 2.7 with acetic acid, a new product in the neutral fraction became predominant (38% by HPLC) while the yields of **3** and **4** fell to 19% and 11% and almost none of the 13-dihydro derivatives were formed. When 1 molar equiv of NaCN was added at this pH, the HPLC yield of the new product was raised to 53%, with further decreases in **3** and **4**.

Further chromatographic workup on silica gel with reverse-phase HPLC analysis showed that this product existed in three isomeric forms, with identical mass spectra, and designated **6A**, **6B**, and **6C**. Most chromatographic fractions were mixtures, but small samples enriched in **6B** and **6C** were also obtained. The near identity of the UV–visible spectra with those of doxorubicin or **1**–**4** indicated again there was no change in the chromophore. The identification of the several fractions all as having structure

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