The preparation of tetrapeptides Boc-Trp-X-Asp-Phe-NH_2 (X = Ala (7), Nle (8), Pro (9), and 3PP (10)) was accomplished by using standard solution methods. For the condensation of Boc-Trp-OH with imino nitrogens, symmetrical anhydride couplings with benzyl ester side chain protection at Asp were used. After Asp deprotection by catalytic hydrogenolysis, the final products were purified by silica gel chromatography (EtOAc/pyridine/H_2O/HOAc systems) and characterized by mass spectrometry, NMR, and combustion analysis. Radioligand binding assays were conducted as described previously.[16,17]

Binding affinities for Boc-CCK_4 and 7-10 to membranes from guinea pig cortex, which contain CCK-B receptors, and guinea pig pancreatic acini, which contain CCK-A receptors, are shown in Table I. The data for cortical receptors indicates the dramatic enhancement in affinity for the CCK-B receptor of the 3PP analogue 10 compared with the Nle analogue 8 and, particularly, the Pro analogue 9. With regard to functional activity, compound 10 is a full agonist relative to CCK_8 and Boc-CCK_4 in stimulating calcium mobilization in NCI-H345 cells, which express CCK-B/gastrin receptors.[17]

It is interesting to consider the activity of 10 in terms of the individual contributions by the *n*-propyl side chain and the constraint imposed by the proline ring of the 3PP residue. Relative to Ala analogue 7, the addition of the *n*-propyl substituent to give Nle analogue 8 results in a 60-fold improvement in binding affinity to the CCK-B receptor, whereas incorporation of the bridging ethylene unit to give Pro analogue 9 results in a 5-fold improvement in binding affinity. The combination of the two modifications results in a 2000-fold improvement in binding affinity compared to 7, ca. 7-fold higher than might be expected on the basis of results for the individual modifications.[18] It is interesting to note that incorporation of *N*-methyl residues at this position in similar tetrapeptide and pentapeptide series has a similarly beneficial effect on receptor binding relative to the corresponding unmethylated analogues.[19,20] The conformational restrictions imposed by the 3PP residue provide useful information on the bioactive conformation of CCK at this receptor; in particular, the ϕ and χ_1 angles of 3PP are restricted to a narrow range, which should closely approximate the angles found in the same region of CCK_4 when it is bound to this receptor.

Largely by virtue of its higher affinity for the CCK-B receptor, compound 10 shows substantially improved cortical selectivity (ca. 1400-fold) relative to Boc-CCK_4 (ca. 74-fold). Incorporation of N-methylated residues at the corresponding position in other CCK analogues also improves selectivity for the cortical receptor.[20-22] Cyclic analogues of CCK_7 and CCK_8 with high selectivity for the B receptor also have been described recently.[23,24]

In related work which will be described separately, we have incorporated 3PP into sulfated heptapeptide analogues of CCK to obtain analogues that bind potently to both pancreatic and cortical CCK receptors.

Acknowledgment. We thank David Whittern and the Abbott Analytical Services Group for providing helpful NMR spectroscopy and other analytical support.

Supplementary Material Available: Full experimental details (5 pages). Ordering information is given on any current masthead page.

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Dual Antagonists of Platelet Activating Factor and Histamine. Identification of Structural Requirements for Dual Activity of *N*-Acyl-4-(5,6-dihydro-11*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridin-11-ylidene)piperidines[1]

Platelet activating factor (PAF)[2] is a biologically active ether phospholipid which is released from a variety of cells[3] involved in the pathogenesis of the allergic and inflammatory response. It produces a variety of biological effects including bronchoconstriction, chemotaxis, and vascular permeability.[3] Consequently, it has been implicated as a mediator in a variety of respiratory and inflammatory diseases. Furthermore, PAF may play a major role in asthma,[4] especially since it has been shown to cause bronchial hyperreactivity in man,[5] a common characteristic of this disease.[6]

(1) This work was presented in part at the Third International Conference On Platelet-Activating Factor and Structurally Related Alkyl Ether Lipids, Tokyo, Japan, May 1989; I2, and at the 198th National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Miami, FL, September 1989; MEDI 56 and 88.
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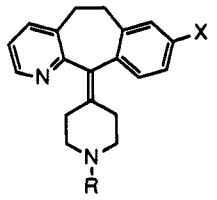
Chart I



1 a X = H, R = CH_3

b X = Cl, R = $CO_2CH_2CH_3$

A number of other mediators are also released at various stages of the allergic response. Although the physiological effects of each of these mediators are relatively well known, the exact role they each play during the response remains unclear. For example, histamine contracts smooth muscle and produces edema,[7] but for the most part the H_1-antihistamines have been ineffective in the treatment of asthma.[8]

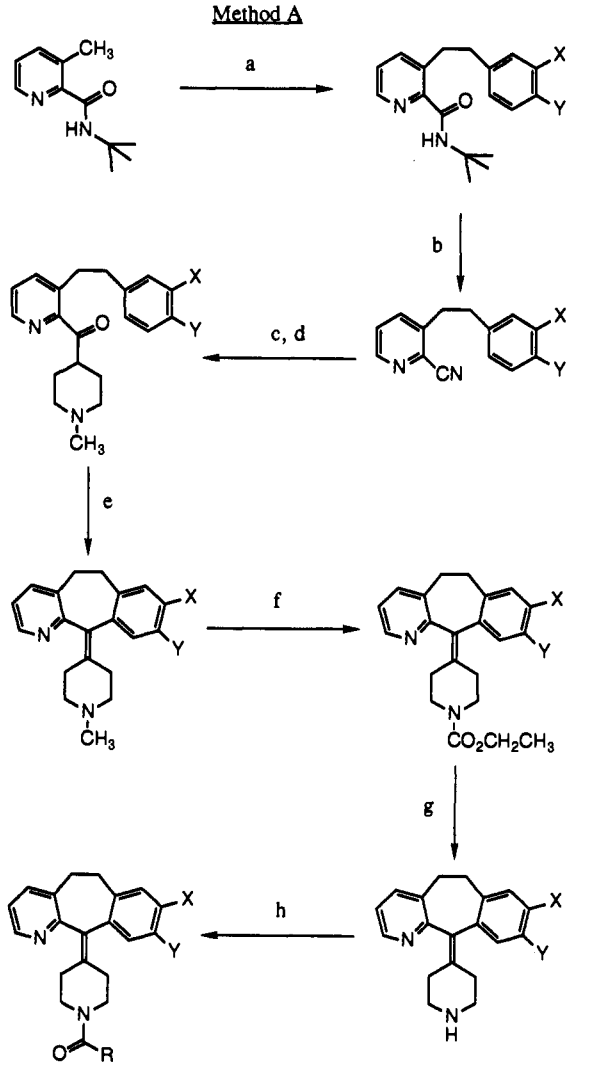
PAF and histamine complement each other during an inflammatory or allergic response. Histamine is preformed in the cell and has a rapid onset of action,[9] whereas PAF is synthesized on demand and often has a slow onset.[3] Both histamine and PAF are released during the asthmatic response, and PAF is believed to play a major role in the late phase.[10] Furthermore, each mediator causes the release of the other in certain cells and tissues.[11]

Recently an intensive effort has been made to discover antagonists of PAF,[3,12] and presently several of them are undergoing clinical evaluation. However, since multiple mediators are involved in these diseases, an agent which inhibits the actions of histamine in addition to those of PAF may be clinically more effective. Herein we report on the synthesis and biological properties of a series of compounds which antagonize the action of both of these mediators. To our knowledge these compounds are the first chemical entities which combine this dual activity in one molecule.

Chemistry

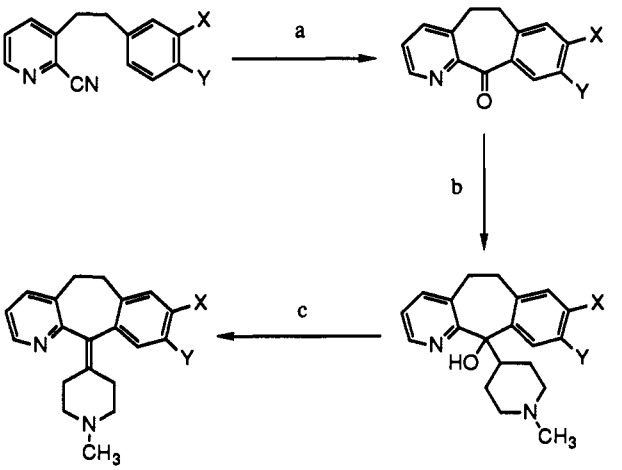
Most of the compounds in Table I were prepared from the corresponding *N*-methylpiperidines. Demethylation was usually accomplished by initial conversion of the *N*-methylpiperidine to the corresponding ethyl (or 2,2,2-trichloroethyl) carbamate followed by hydrolysis (Zn/AcOH/Δ for 2,2,2-trichloroethyl) to the unsubstituted piperidine (steps f and g, Scheme I). Acylation using either the appropriate acid chloride or anhydride (ethyl

Scheme I[a]



[a] (a) 2 equiv *n*-BuLi, THF, -40 °C, then *m*-X-*p*-Y-$C_6H_4CH_2Cl$; (b) $POCl_3$, Δ; (c) $ClMgC_5H_9NCH_3$, THF, Δ; (d) HCl, H_2O; (e) CF_3SO_3H or PPA, Δ; (f) $ClCO_2CH_2CH_3$, $C_6H_5CH_3$, Et_3N, Δ; (g) KOH or HCl, H_2O, Δ; (h) RCOCl or $(RCO)_2O$, C_5H_5N, CH_2Cl_2.

Scheme II[a]



[a] (a) PPA or CF_3SO_3H, Δ; (b) $ClMgC_5H_9NCH_3$, THF, -40 °C; (c) H_2SO_4 or CF_3SO_3H.

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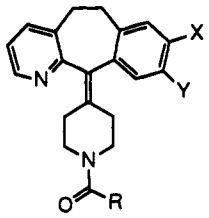
Table I. In Vitro PAF Antagonist and H_1 Binding Activities[a] of *N*-Acyl-4-(5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidines



compound	R	X	Y	method of synthesis	PAF antagonist[b] IC_{50}, μM	H_1 binding[c] K_i, μM
1a (azatadine)		H	H		>50	0.0039 ± 0.0022
1b (loratadine)	OCH_2CH_3	Cl	H	A	>50	0.35 ± 0.13
2	H	Cl	H	A	13 ± 2[e]	0.021 ± 0.006[e]
3	CH_3	Cl	H	A	0.61 ± 0.05[d]	0.32 ± 0.09[e]
4	CH_2CH_3	Cl	H	A	2.4 ± 1.1	0.048 ± 0.022[e]
5	$CH_2CH_2CH_3$	Cl	H	A	33 ± 16	0.051 ± 0.030[e]
6	$C(CH_3)_3$	Cl	H	A	>50	0.081 ± 0.056[e]
7	C_6H_5	Cl	H	A	>50	0.19 ± 0.07[e]
8	CH_3	H	H	B	1.4 ± 0.3	2.0 ± 0.8
9	CH_3	H	Cl	B	3.4 ± 0.8	1.5 ± 0.1
10	CH_3	Cl	Cl	A	1.8 ± 0.1	3.1 ± 0.1
11	CH_3	F	H	A	0.67 ± 0.03	0.72 ± 0.18
12	CH_3	H	F	B	0.82 ± 0.07	2.9 ± 1.0
13	CH_3	F	F	B	1.0 ± 0.2	10 ± 5
14	CH_3	Br	H	A	0.81 ± 0.09	
15	CH_3	CH_3	H	A	1.0 ± 0.5	0.19 ± 0.01
16	CH_3	OCH_3	H	A	3.4 ± 0.3	1.6 ± 0.4
17[f]	CH_3	OH	H	A	8.7 ± 1.8	>6.3
18a (*Z* isomer)				B	>50	0.85 ± 0.15
18b (*E* isomer)				B	>50	0.29 ± 0.02
19a					28 ± 5	>6.3
20				B	41 ± 2[e]	5.0 ± 2.2
21				B	>50	1.2 ± 0.2
WEB 2086					0.04 ± 0.005[e]	
L-652,731					1.5 ± 0.5[e]	
chlorpheniramine					>50	0.0055 ± 0.0011[e]

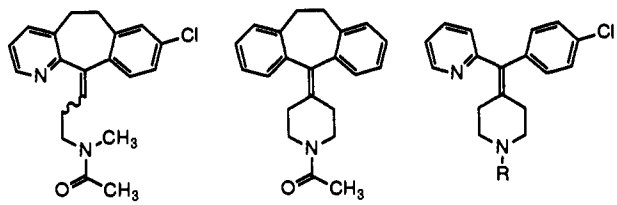
[a] Unless otherwise noted the values represent the mean of two independent experiments with the associated errors representing the range from the mean. [b] Values are a measure of the concentration of drug required to cause a 50% inhibition of PAF-induced platelet aggregation of human platelet-rich plasma when challenged with 25 nM PAF.[21] [c] Values were determined by using a receptor binding assay using rat brain membranes and the experimentally determined value of 2.7 nM for the K_D of [^{3}H]pyrilamine.[21] [d] Value is the mean ± the standard error of the mean for 11 independent experiments. [e] Value is the mean ± the standard error of the mean for three to eight independent experiments. [f] Phenol 17 was obtained from methoxy ether 16 by cleavage of the methyl ether using BBr_3/CH_2Cl_2.

formate for **2**) provided the target compounds (**2–20**).

The corresponding *N*-methylpiperidines were prepared via one of two routes. Method A (Scheme I) was developed by Schumacher and co-workers for the synthesis of loratadine (**1b**)[13] (Chart I). This sequence centers on building the appropriate appendages into the molecule and then cyclizing it to provide the central seven-membered ring. Method B (Scheme II) is a modification of an earlier route[14] which centers on the formation of the seven-membered ring before the addition of the piperidine ring. Method A was preferred, but failed to provide the desired cyclized product in a number of cases (see Table I).

Compounds **18a,b** (Chart II) were prepared via method B using the Grignard reagent derived from 3-chloro-*N*,*N*-dimethylpropylamine. Compound **20** was also synthesized via method B using dibenzosuberone as substrate. The nonbridged derivative **19a** was prepared from the corresponding *N*-methylamine **19b**[15] by employing the demethylation–acylation sequence described above. Ketone

Chart II



18 a Z-isomer b E-isomer **20** **19** a R = $COCH_3$ b R = CH_3

21 was synthesized as illustrated in Scheme III.

Discussion

Azatadine (**1a**)[14,16] and loratadine (**1b**)[17,18] (Chart I) are two H_1-antihistamines which are presently in clinical use. Both are very weak PAF antagonists as demonstrated by

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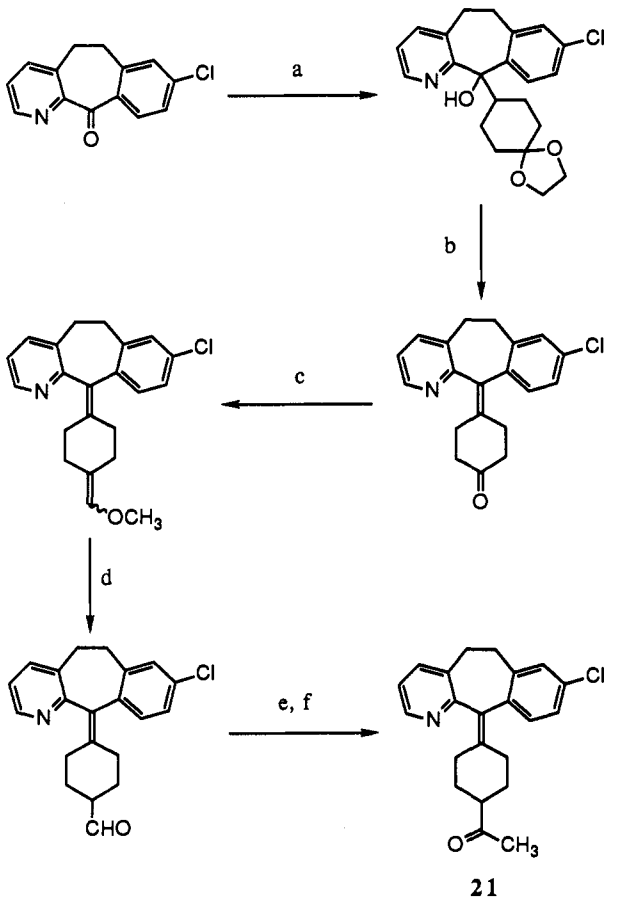
Scheme III[a]



21

[a] (a) $BrMgC_6H_9(O_2C_2H_4)$, Et_2O, THF; (b) H_2SO_4, H_2O; (c) $(C_6H_5)_3P{=}CHOCH_3$, THF; (d) H_2SO_4, THF, H_2O; (e) CH_3MgBr, THF, -78 °C; (f) PDC, CH_2Cl_2, 0 °C.

their inability to inhibit PAF-induced aggregation of human platelet-rich plasma at compound concentrations of 50 μM and below (Table I). Several compounds were prepared with different functionalities on the piperidine nitrogen; only the amide derivatives exhibited appreciable anti-PAF activity.[19]

Among simple alkyl amides (i.e. **2–7**), the anti-PAF activity appears to be optimal for acetamide **3** (Table I). Removing a methylene group from the acetamide moiety, or adding one to it, modestly reduced the activity (cf. **2** and 4 with **3**). Large amides (e.g. **6** and **7**) were essentially inactive. It is interesting to note this trend in antagonist potencies parallels the relative trend in agonist activities for C-16-PAF and analogues which contain these same groups at the C-2 position.[20] As is evident from the H_1 binding data, the size of the amide has a varying effect on the antihistamine activity of these derivatives.

Substitution of the aromatic ring raises several interesting points. First, removal of the chlorine atom from the C-8 (X) position results in modest loss of both the anti-PAF and antihistamine activity (**3** vs. **8**). This finding is confirmed in vivo for the anti-PAF activity (Table II) and is also consistent with the antihistamine SAR for loratadine (**1b**).[17] Furthermore, placement of a chlorine atom at C-8 (X) appears to be optimal for both activities since

Table II. Selected in vivo Anti-PAF and Antihistamine Activities[a] of *N*-Acyl-4-(5,6-dihydro-11*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridin-11-ylidene)piperidines in Guinea Pigs

	% inhibn of guinea pig bronchospasm[b] (10 mg/kg orally)			
	PAF		histamine	
compd	*N*[c]	% inhibn	*N*[c]	% inhibn
3	4	70 ± 6	4	99 ± 1
4	4	45 ± 8[d]		
8	4	44 ± 9	2	96 (98, 94)[e]
9	4	14 ± 17		
10	2	14 (20, 9)[e]		
11	3	44 ± 13	3	79 ± 6[f]
12	4	39 ± 7		
13	4	54 ± 12	3	0
14	4	74 ± 13	3	99 ± 1
15	4	32 ± 19		
16	2	0		
WEB 2086	2	99 (100, 98)[e]	4	7 ± 15
WEB 2086	5	60 ± 11[g]		

[a] Compounds administered 2 h (po) before iv challange with 0.4 μg/kg of PAF or 10 μg/kg of histamine.[21] [b] Value is the mean ± the standard error of the mean unless otherwise noted. [c] *N* represents the number of animals used in each determination. [d] Dose was 15 mg/kg. [e] Number in parentheses represents the individual % inhibitions. [f] Dose was 3 mg/kg. [g] Dose was 1 mg/kg.

the C-9 (Y) isomer **9** and the dichloro derivative **10** are slightly weaker.

The dissimilarity between the PAF and histamine receptors becomes apparent when one compares the activities of the various aryl substituted derivatives. The PAF receptor appears to prefer a relatively hydrophobic substituent since the halogen and alkyl derivatives are approximately equipotent in the platelet-aggregation assay (cf. **3**, **11**, **14**, **15**). The more polar methoxy and hydroxy derivatives **16** and **17** are somewhat weaker. The activity of these compounds in the H_1 binding assay varies considerably even among the hydrophobic analogues. For example, both the chloro and difluoro derivatives **3** and **13** are approximately equipotent as PAF antagonists, but the difluoro compound **13** is a much weaker antihistamine both in vitro (Table I) and in vivo (Table II). Hence, one can utilize substitution in the aromatic ring to adjust the desired amount of antihistamine activity in this series.

Conformational rigidity is important for optimum anti-PAF activity. The less constrained analogues **18a,b** (broken piperidine ring) and **19a** (ethylene bridge removed) are weak antagonists of PAF. Furthermore, anti-PAF activity requires both nitrogen atoms. Bis-aryl derivative **20**, which lacks the pyridine nitrogen, was a weak antagonist, while compound **21**, which lacks the nitrogen in the piperidine ring, was completely inactive at a dose of 50 μM.

In vivo, many of the more potent compounds were active orally against PAF- and histamine-induced bronchospasm in guinea pigs (Table II). In general, their potencies parallel their respective in vitro activities. Because of these and other accumulated data, compound **3** (SCH 37370) has been selected for clinical evaluation. Compound **3** inhibited both PAF- and histamine-induced bronchospasm in guinea pigs with oral ED_{50} values of 6.0 and 2.4 mg/kg, respectively. The complete pharmacological profile of this compound has recently been reported elsewhere.[21]

Acknowledgment. We thank Drs. Frank J. Villani and Charles V. Magatti for the preparation of compounds **2** and

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Folic Acid Analogues Lacking the 2-Carbon Are Substrates for Folylpolyglutamate Synthetase and Inhibit Cell Growth

Recent reports from several laboratories have pointed to the fact that folylpolyglutamate synthetase (FPGS), the enzyme responsible for the conversion of endogenous reduced folates as well as a variety of folate antagonists to their γ-polyglutamylated forms,[1] has a high degree of side chain specificity but is relatively tolerant of structural changes in rings A and B, the "bridge region" and the phenyl ring (Figure 1). With regard to ring A, for example, it has been shown that replacement of the 2-amino group by hydrogen in the N^{10}-methyl-, N^{10}-ethyl-, N^{10}-allyl-, and N^{10}-propargyl-substituted derivatives of 5,8-dideazafolic acid results in a minimal change in either the K_m or V_{max} for γ-diglutamate formation by mouse liver FPGS.[2] Similar results were obtained for replacement of the 2-amino group by hydrogen in 10-oxafolic acid and 10-thiafolic acid,[3] and for replacement of the 2-amino group by hydrogen or methyl in aminopterin.[4] We postulated that since changes in the 2-substituent at in ring A are well tolerated, *the carbon itself at position 2 may not be absolutely required for binding to the enzyme.* To test this hypothesis we prepared the heretofore undescribed compound *N*-[4-[[(2-amino-3-carbamoylpyrazin-5-yl)methyl]-amino]benzoyl]-L-glutamic acid (1), which may be viewed as a folic acid analogue in which *both the 2-amino group and C_2 have been deleted.* Also synthesized was *N*-[4-[[(2-amino-3-carbamoylpyrazin-5-yl)methyl]formamido]-benzoyl]-L-glutamic acid (2), the corresponding ring-opened analogue of N^{10}-formylfolic acid. Compound 1 inhibited the growth of L1210 murine leukemic cells in culture with an IC_{50} of 5 μM and was comparable to folic acid as a substrate for FPGS, providing the first demonstration that an intact A ring in folate analogues is not an absolute requirement for FPGS substrate activity. We believe these results are a timely and potentially exploitable lead for the design of other biologically active folate analogues.

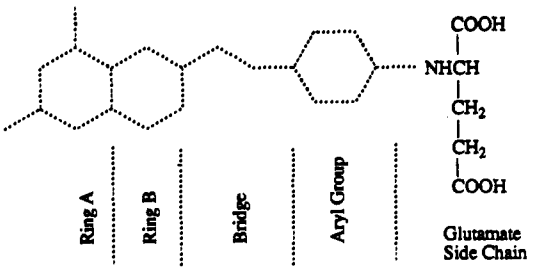
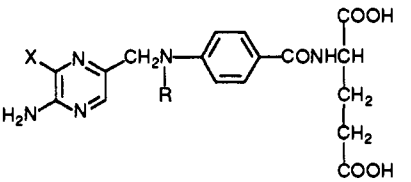


Figure 1. General structure of FPGS substrates.



1: X = $CONH_2$, R = H
2: X = $CONH_2$, R = CHO
3: X = CN, R = H

The starting material 3 was prepared previously in our laboratory by N-monoalkylation of di-*tert*-butyl *N*-(4-aminobenzoyl)-L-glutamate with 2-amino-5-(chloromethyl)pyrazine-3-carbonitrile followed by cleavage of the ester groups with TFA.[4,7] Conversion of 3 to the carboxamide 1 was accomplished in quantitative yield by allowing a solution of the nitrile in 1 N NaOH to stand at room temperature and monitoring the course of reaction by HPLC. Complete conversion was observed after 2.5 h, and the product was purified by ion-exchange chromatography (Dowex 50W-X2, H^+ form) using 3% NH_4OH as the eluent. Freeze-drying afforded analytically pure 1 as a sesquihydrate [HPLC 30 min (C_{18} silica gel, 5% MeCN in 0.1 M NH_4OAc, pH 5.0, 2 mL/min); UV λ_{max} (pH 7.4) 253 nm (ε 15 500), 289 (19 400), 357 (6710), λ_{max} (0.1 N HCl) 216 nm (ε 13 900), 246 (15 900), 294 (10 400), 358 (6620)].[8]

Reaction of 1 with 95% formic acid (room temperature, 7 days) and purification by ion-exchange chromatography (DEAE-cellulose, HCO_3^- form) using H_2O followed by 0.2 and 0.4 M NH_4HCO_3 as the eluents afforded a 65% yield of 2 as a hydrated partial ammonium salt [HPLC 7 min (C_{18} silica gel, 5% MeCN in 0.1 NH_4OAc, pH 7.5, 1 mL/min); NMR (D_2O with enough K_2CO_3 to dissolve the sample) δ 7.33 (d, J = 8 Hz, $C_{3'}$- and $C_{5'}$-H), 7.78 (d, J = 8 Hz, $C_{2'}$- and $C_{6'}$-H), 8.37 (minor) and 8.50 (major) (singlets, NCHO rotomers); UV λ_{max} (pH 7.4) 253 nm (ε 26 700), 357 (5980), λ_{max} (0.1 N HCl) 249 nm (ε 18 700), 359 (4520), λ_{max} (0.1 N NaOH) 253 nm (ε 27 500), 357 (6250)].[8] HPLC evidence for two other minor products was obtained, one of which appears to be formed from 1, but no attempt was made to isolate and characterize these side products. The site of the N-formylation in 2 was determined to be N^{10} as opposed to the 2-amino group on the basis of the ^1H NMR spectrum, which showed the $C_{3'}$ and $C_{5'}$ protons as a doublet at δ 7.33, whereas the chemical

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(8) Combustion analyses for 1 and 2 were within ±0.4% of theoretical values for the empirical formulas $C_{18}H_{20}N_6O_6 \cdot 1.5H_2O$ and $C_{19}H_{20}N_6O_7 \cdot 0.2NH_3 \cdot 1.25H_2O$, respectively.

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