Synthesis of Monoamides of Methotrexate from L-Glutamic Acid Monoamide t-Butyl Esters

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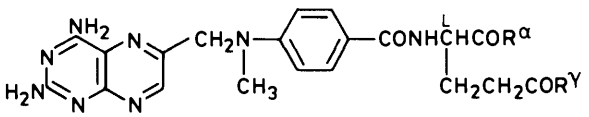
Analogues of methotrexate (amethopterin) (**1**) with α- or γ-monoamide functions [*viz.* the α- and γ-primary amides (**16a**) and (**26a**); the following *N*-substituted amides: α-methyl-, α-ethyl-, α- and γ-propyl-, α-isopropyl-, α-butyl-, α-isobutyl-, α-sec-butyl-, α-t-butyl, α-benzyl-, and α- and γ-cyclohexylamide (**16b**—**d**), (**26d**), (**16e**—**k**), (**26k**) respectively; and the *NN*-disubstituted amides: γ-piperidide (**26l**) and γ-morpholide (**26m**)] were synthesized starting with t-butyl L-isoglutamine (**12a**), t-butyl L-glutamine (**22a**), or the appropriate *N'*-alkyl or *N'N'*-dialkyl analogues (**12b**—**k**), (**22d**), (**22k**), (**22l**), and (**22m**). The corresponding *N*-benzyloxycarbonyl compounds (**11**) and/or (**21**) from which the above L-glutamic acid derivatives were obtained were generally synthesized by mixed-anhydride coupling of *N*-benzyloxycarbonyl-L-glutamic acid (**9**) with the appropriate amine, conversion into the t-butyl ester, and chromatographic separation. The resulting α-monoamide γ-t-butyl ester (**11**) and γ-monoamide α-t-butyl ester (**21**) are unambiguously distinguished by mass spectrometry and ^{13}C n.m.r. spectroscopy. Factors which affect the γ-amide/α-amide product ratio are discussed. The *N*-deprotected L-glutamic acid monoamide t-butyl esters (**12**) or (**22**) were individually coupled to *N*-trifluoracetyl-*p*-methylaminobenzoic acid, and the resulting α- or γ-monoamide t-butyl esters (**13**) or (**23**) of *N*-(*p*-methyl (trifluoroacetyl)aminobenzoyl)-L-glutamic acid was hydrolysed. The *N*-deprotected product, *viz.* t-butyl *N*-(*p*-methylaminobenzoyl)-L-glutamate α- or γ-monoamide (**14**) or (**24**) was converted into the appropriate methotrexate-monoamide t-butyl ester (**15**) or (**25**), and thence the desired methotrexate-monoamide (**16**) or (**26**), by reaction with 2,4-diamino-6-bromomethylpteridine (**17**) or by the Taylor procedure. Features of the mass and ^{13}C n.m.r. spectra of the intermediates are discussed.

Methotrexate or amethopterin, N-[*p*-(2,4-diaminopteridin-6-yl)methyl(methyl)aminobenzoyl]-L-glutamic acid (**1**), is an anti-metabolite widely used in the control of acute leukaemia and other neoplastic conditions.[1] In this work a series of 11 α-monoamide analogues of methotrexate [*viz.* α-monoamides (**16a**—**k**)] has been synthesized for evaluation as latent forms of the parent drug under conditions characterized by high activities of proteolytic enzymes in proliferating cells.[2] Also synthesized for comparison are 5 γ-monoamides (**26a**), (**26d**), (**26k**), (**26l**), and (**26m**). Some products are being used for n.m.r. studies[3] of interactions with dihydrofolate reductase, the target enzyme.



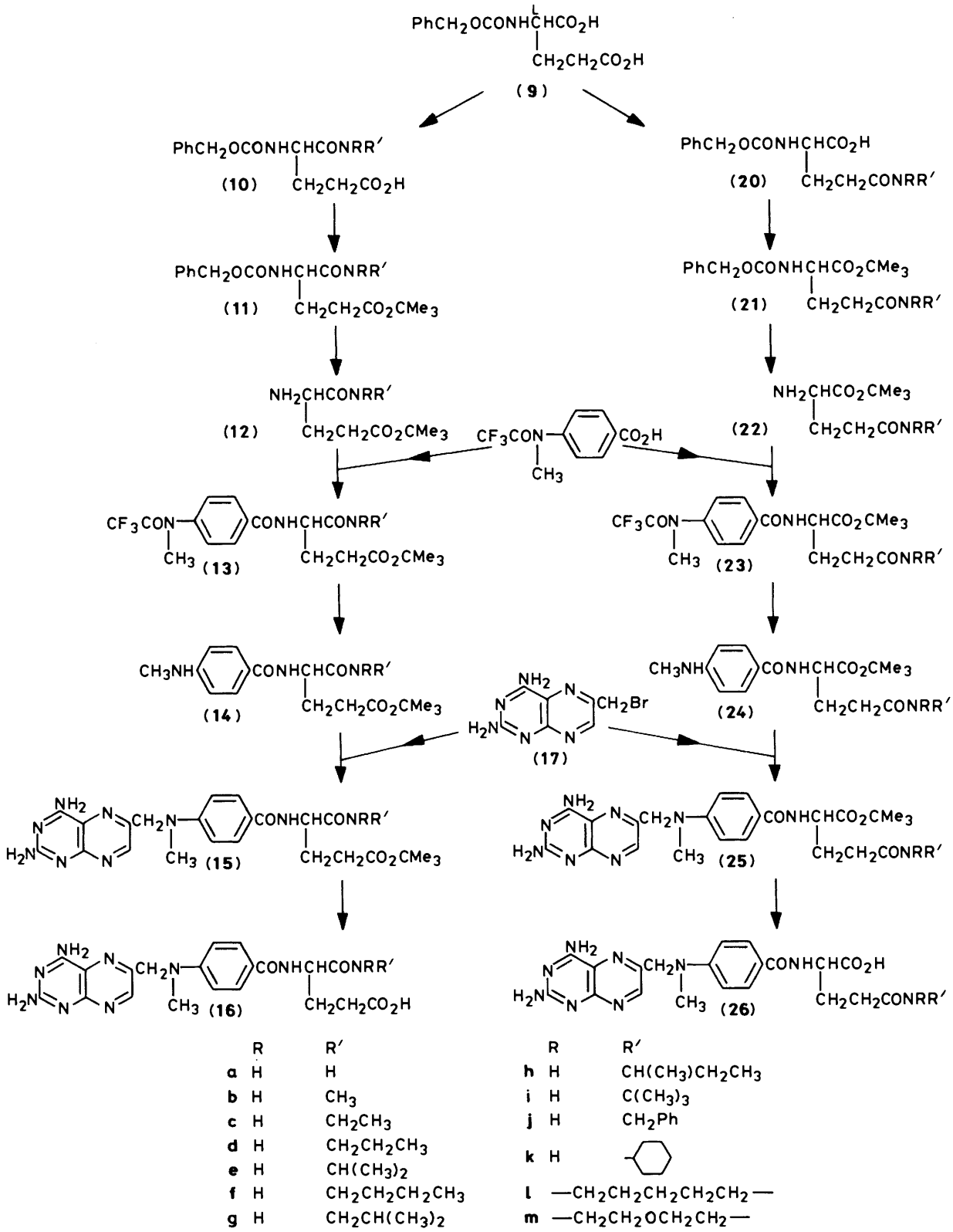
	R^{α}	R^{γ}
(**1**)	OH	OH
(**2**)	$NHCH_2Ph$	$NHCH_2Ph$
(**3**)	NH_2	NH_2
(**4**)	NHPh	NHPh
(**5**)	OH	$NHCH_2CH_2CH_2CH_3$
(**6**)	OH	$NHCH_2Ph$
(**7**)	OH	$NHCH_3$
(**8**)	OH	$NHCH_2CH_2CH_2CH_2CH_3$

Two types of simple amide derivatives prepared by earlier workers fail to meet our requirements. Thus bisamides such as (**2**)—(**4**)[4] lack a free carboxylic group needed for folate-type active transport,[5] while γ-monoamides such as (**5**), (**6**),[6] (**7**) and (**8**)[7] bind tightly to dihydrofolate reductase, and are expected to be as damaging to non-proliferating cells as methotrexate itself. In contrast, α-monoamides synthesized in this work are significantly weaker inhibitors[2,7] before being converted into the active drug. Only one simple α-monoamide of methotrexate, *viz.* the primary amide (**16a**), had been synthesized previously,[7] although conjugation at the α-carboxylate group to α-amino acids had been carried out.[7]

The unequivocal synthesis of α- and γ-monoamides of methotrexate requires a build-up from the appropriately protected L-glutamic acid α- or γ-monoamide, and the use of methods of peptide synthesis. We have adopted a convenient entry to both α- and γ-monoamides of methotrexate, consisting of careful separation of a mixture of t-butyl esters (**11**) and (**21**) of *N'*-substituted *N*-benzyloxycarbonyl-L-isoglutamine and -L-glutamine. These α- and γ-monamide t-butyl esters (**11**) and (**21**) were obtained *via* mixed carbonic–carboxylic anhydride coupling of *N*-benzyloxycarbonyl-L-glutamic acid (**9**) with one equivalent[8] of the appropriate primary or secondary amine; the resulting mixture of the α- and γ-monoamide (**10**) and (**20**) was esterified directly to convert the remaining free carboxylic acid group into the t-butyl ester.† Details of the coupling reaction are discussed later. Thereafter the α- and γ-monoamide series were dealt with *separately* and in parallel as is summarized in Scheme 1 [α-series: (**9**) ⟶ (**16**); γ-series: (**9**) ⟶ (**26**)] and in Scheme 2 [α-series: (**14**) ⟶ (**16**); γ-series: (**24**) ⟶ (**26**)]. The crucial structural distinction between the α- and γ-monoamides is discussed further below.

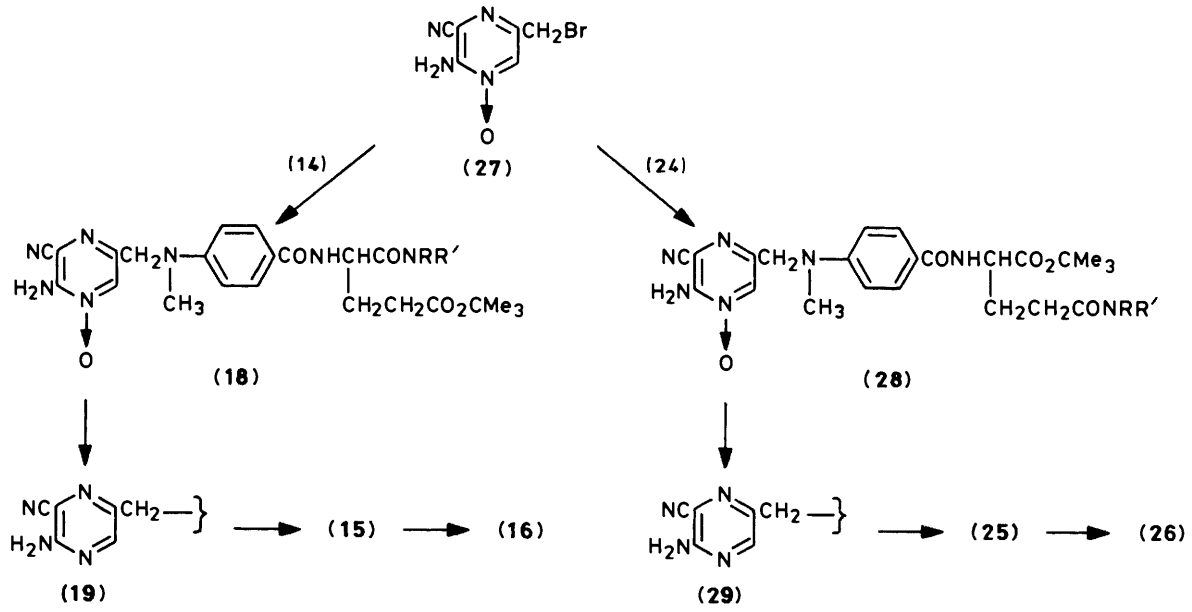
The present method combines the protection of the L-glutamic acid monoamide moiety as the acid-labile t-butyl ester[9] (which is not removed until the complete methotrexate skeleton is assembled) with blocking of the *p*-methylaminobenzoyl portion as the *N*-trifluoroacetyl derivative (which is base labile).[10] Use of the t-butyl ester avoids α ⟶ γ transpeptidation reactions which take place (*via* the cyclic imide) during alkaline hydrolysis of the methyl or ethyl esters (say) of *N*-protected

† In some cases the separation into α- and γ-monoamides could be effected by crystallization prior to ester formation.



Scheme 1.

glutamic acid monoamides.[11] Unprotected *p*-methylaminobenzoic acid is weakly acidic and couples poorly to amines,[12] but protection by the electron-withdrawing *N*-trifluoroacetyl group strengthens the acid and facilitates coupling to a glutamic acid derivative.[13] Thus for example *N*-trifluoroacetyl-*p*-methylaminobenzoic acid was coupled to L-glutam-α-propylamide (*N'*-propylisoglutamine) t-butyl ester (**12d**) [obtained by hydrogenolysis[14] of *N*-benzyloxycarbonyl-L-glutam-α-propylamide (*N*-benzyloxycarbonyl-*N'*-propyl-L-isoglutamine) t-butyl ester (**11d**)] to yield the *N*-trifluoroacetyl-protected 'dipeptide'



Scheme 2.

Table 1. Methane chemical ionization mass spectra[a] of α- and γ-monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate

(a) *α-Monoamides*	Ion								
Compound	MH^{+}[b]	a[b]	b	f	e	g	d	j	h
(11a)	337	*281**	*236*	*173*	*237*		*219*	*147*	229
(11b)	*351*	*295*	*236*	*187**	251	*169*	233		243
(11c)	*365*	*309**	*236*	*201*	265	*183*	*247*	175	
(11d)	*379*	*323**	*236*	*215*	279	*197*	261	189	
(11e)	*379*	*323**	236	*215*	279	197	261	189	
(11f)	*393*	*337**	236	*229*		211	275		
(11g)	*393*	*337**	*236*	*229*		*211*	275	203	
(11h)	*393*	*337**	*236*	*229*		*211*	275	203	
(11i)	*393*	*337**	236	*229*	293		*275*	203	285
(11j)	*427*	*371**	*236*	*263*	327	*245*	309	237[c]	319
(11k)	*419*	*363**	*236*	*255*	319	237	301	*229*[c]	*311*
(11l)	*405*	349	236	241			287	215	297
(11m)	*407*	*351**	*236*	*243*	307		289	217	*299*
(b) *γ-Monoamides*									
Compound	MH^{+}[b]	a[b]	c	f	e	g	i	j	h
(21a)	*337*	*281**	235	*173*	*237*		203	*147*	
(21b)	*351*	*295**	249	*187*	*251*			161	
(21c)	*365*	*309**	263	*201*	*265*	183		175	
(21d)	*379*	*323**	*277*	*215*	*279*	197		*189*	
(21e)	*379*	*323**	277	*215*	*279*			189	
(21f)	*393*	*337**	291	*229*	*293*			203	
(21g)	*393*	*337**	291	*229*	293			203	
(21h)	*393*	*337**	291	*229*	293			203	
(21i)	*393*	*337**	291	*229*	*293*			203	
(21j)	*427*	*371**	325	*263*	327	245	293	*237*	
(21k)	*419*	*363**	*317*	*255*	319	237	*285*	*229*	311
(21l)	*405**	*349*	303	*241*			*271*	*215*	297
(21m)	*407*	*351**	*305*	*243*	307		273	*217*	299

[a] Recorded as *m/z* in a.m.u. Italicized peaks are of abundance 10% or above, and unitalicized ions less than 10%; base peaks are italicized and asterisked. For origin of ions *a*—*j*, see text. [b] The C_2H_5 adduct ions of *M* and $M - C_4H_8$ usually observed. [c] Ion *i* of *ca.* 1/5th abundance of *j* also observed.

Table 2. Helium charge-exchange mass spectral data[a] of α- and γ-monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate

(a) α-*Monoamides*	Ion									
Compound	$M^{+\cdot}$	$M - C_4H_8$	*b*	$b - CO_2$	b – PhCH	$PhCH_2^+$	*f*	*d*	*g*	*k*
(11a)		280	*236*	*192**	146	*b*	*173*	*219*	*155*	*145*
(11b)		294	236	192	146	*91**	187	233	169	159
(11c)			236	192	146	*91**	201	247	183	173
(11d)	378		236	*192*	146	*91**	215	261	197	187
(11e)			236	*192*	*146*	*91**	215	261		187
(11f)			*236*	*192*	146	*91**	229	275	211	201
(11g)			236	192	146	*91**				
(11h)			236	192	146	*91**				
(11i)			236	*192*	*146*	*91**	229	275		
(11j)	426	370	236	*192*	146	*91**	263	309		
(11k)			236	*192*	*146*	*91**				
(11l)			236	192		*91**				
(11m)			236	192	146	*91**	243	289	225	

(b) γ-*Monoamides*										
Compound	$M^{+\cdot}$	$M - C_4H_8$	*c*	$c - PhCH_2$-OCONH$_2$	*a* – PhCH	$PhCH_2^+$	*f*	unassigned[c]	$k - CO_2$	*k* (or *c* – PhCH)
(21a)		280	235	84	191	*91**	173	174	101	145
(21b)			249	98		*91**		174	115	159
(21c)			263	*112*	219	*91**	201	174	129	173
(21d)		322	277	126	233	*91**	215	174	143	187
(21e)			277	126	233	*91**	215	174	143	187
(21f)			291	*140*	247	*91**	229	174	157	201
(21g)			291	140		*91**	229	174	157	201
(21h)			291	140		*91**		174	157	
(21i)		336	291	140	247	*91**	229	*174*	157	201
(21j)	426		325	*174*[d]	281	*91**		*174*[d]	191	235
(21k)		362	317	166	273	*91**	255	174	183	227
(21l)			*303*			*91**		174	169	
(21m)		350	305		261	*91**	243	174	171	215

[a] See footnote *a* of Table 1. [b] Ions of $m/z < 100$ were not recorded. [c] Mass matching under electron impact on **(21i)**, **(21l)**, and **(21m)** indicated a formula of $C_{11}H_{12}NO$. [d] There are two possible origins of this ion.

(13d).* Couplings were achieved by the mixed carbonic–carboxylic anhydride method,[15] or (since the activated carbonyl component is non-chiral) *via* the acid chloride.

Though aqueous alkali is traditionally used to remove the *N*-trifluoroacetyl group,[10] an alternative reagent was needed to remove this group from the protected 'dipeptides' **(13)**, due to the latter's insolubility in aqueous solutions. By the use of a mixture of triethylamine, methanol, and water (2:1:1), t-butyl *N*-(*p*-methylaminobenzoyl-L-glutamic acid monoamides [*e.g.* **(14d)**] were smoothly formed.† The product was converted into the appropriate methotrexate-monoamide t-butyl ester [*e.g.* **(15d)**], either directly by displacement on 2,4-diamino-6-bromomethylpteridine **(17)**,[16] or in several steps *via* reaction with 2-amino-5-bromomethyl-3-cyanopyrazine *N*-oxide **(27)**[17] (Scheme 2). Finally, hydrolysis of the t-butyl ester with trifluoroacetic acid yielded the required monoamide of methotrexate, **(16)** or **(26)**, a process monitored by high-pressure liquid chromatography.

Distinction Between α- and γ-Monoamides of t-Butyl L-*Glutamate*.—In this work it is essential to be able to distinguish unequivocally between members of a given pair of α- and γ-monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate [*viz.* **(11)** and **(21)**]. This has been possible since characteristic and consistent differences in ^{13}C n.m.r. and mass spectral data were noted by us.

The methane chemical ionization mass spectral data of 13 pairs of monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate are given in Table 1. A characteristic ion *b* of *m/z* 236 (abundance *ca.* 10%) was found for *all* the α-monoamides **(11a—m)**. The corresponding γ-monoamides **(21a—m)** *all* gave rise instead to ion *c* of mass corresponding to (219 + NRR′) (abundance *ca.* 5%). For both series, the characteristic ion is due to loss of C_4H_8 giving rise to ion *a* (usually the base peak) followed (or accompanied) by cleavage at the C^{α}–CO bond (with hydrogen transfer). Cyclization involving the benzyloxycarbonyl group and the side-chain ester is probably implicated in the formation of an ion *d* which was given by *all* the α-monoamides, but not by any γ-monoamide, and which is formally derived from MH^+ by the loss of ($C_4H_9OH + CO_2$). The helium charge-exchange fragmentation pattern of the same set of monoamides (Table 2) parallels that derived from

* 'Dipeptide' is used here to describe the product of coupling two amino acids, not necessarily α-amino acids.

† There was no evidence that this treatment resulted in racemized *p*-methylaminobenzoylglutamyl derivatives. Thus the 'dipeptide' **(24a)** [from removal of the *N*-trifluoroacetyl group in this manner from *N*-[methyl(*p*-trifluoroacetyl)aminobenzoyl]-L-glutamine t-butyl ester **(23a)**], on sequential addition of the chiral shift reagent tris-[3-(2,2,2-trifluoro-1-hydroxyethylidene)-(+)-camphorato]europium (G. R. Sullivan in 'Progress in Stereochemistry,' eds E. L. Eliel and N. L. Allinger, Wiley, New York, 1978, vol. X, pp. 287—330), yielded a lanthanide-induced ^1H n.m.r. spectrum which showed no evidence of the presence of the enantiomer. Furthermore, using this method cleavage of the trifluoracetyl group of di-t-butyl *N*-[*p*-methyl-(trifluoroacetyl) aminobenzoyl]-L-glutamate, $[\alpha]_D$ −15.5 °C, gave di-t-butyl *p*-methylaminobenzoyl-L-glutamate, m.p. 92—93 °C, with $[\alpha]_D$ −11.2 °C (both *ca.* 2% w/v in methanol) (unpublished results).

Table 3. ^{13}C Chemical shifts of α- and γ-monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate[a]

	α-Monoamides					γ-Monoamides				
	(11a)	(11b—h), (11j), (11k)	(11i)	(11l), (11m)	(12k)	(21a)	(21b—k)	(21l), (21m)	(22d), (22k)	(22m) (protonated)
$OC(CH_3)_3$	27.8	27.8	27.9	27.9	27.7	27.7	27.7	27.7	27.7	27.7
$^{\beta}CH_2CH_2CO$	27.8	27.9[b]	28.4	28.1	30.0	28.1	28.2—28.8	27.7	30.3	25.4
$CH_2{}^{\gamma}CH_2CO$	31.3	31.4	31.5	*30.5/30.2*	31.6	31.4	32.1—32.6[c]	*28.8*	32.6	28.6
$NH^{\alpha}CHCO$	53.8	54.2	54.5	*49.8/49.4*	54.2	54.0	54.0	*54.1*	54.1	52.9
$PhCH_2O$	66.7	66.6	66.8	66.6		66.7	66.7	66.4		
$OC(CH_3)_3$	*80.6*	*80.4—80.6*	*80.8*	*80.4*	80.2	*82.1*	*81.9—82.2*	*81.4/81.9*	80.9	83.7
Ph (CH)	127.7	127.7	127.9	127.8		127.7	127.9	127.7		
	127.8	127.8	128.0	127.8		127.7	127.9	127.7		
	128.2	128.2	128.4	128.2		128.2	128.3	128.1		
Ph (1′)	136.0	136.1	136.2	136.3		136.1	136.1	136.2		
$OC(O)NH$	156.1	156.0	156.0	155.9		156.1	156.1	155.8		
$^{\gamma}CH_2C(O)O$	172.4	172.4	172.7	171.9	172.5					
$^{\alpha}CHC(O)O$						170.9	170.9	170.9	174.6	167.8*
$^{\alpha}CHC(O)NRR'$	174.1	170.3—*171.2*[d]	170.2	169.3/170.0	172.9					
$^{\gamma}CH_2C(O)NRR'$						174.7	170.9—171.8[e]	169.7/170.2	172.1/171.2	169.9*

[a] Tabulated chemical shifts (δ_C) are given in p.p.m. downfield from $SiMe_4$ in $CDCl_3$ (to ± 0.1 p.p.m.), $\delta_C(CDCl_3)$ 76.9 p.p.m. Chemical shifts for amide *N*-alkyl carbons (to ± 0.2 p.p.m.) are: for compounds of the b series (CH_3), 26.0; for c series (CH_2CH_3) 34.1 (α′), 14.4 (β′); for d series ($CH_2CH_2CH_3$), 41.0 (α′), 22.4 (β′), 11.1 (γ′); for e series ($CHMe_2$), 41.3 (α′), 22.3 (β′); for f series ($CH_2CH_2CH_2CH_3$) 39.0 (α′), 31.2 (β′), 19.7 (γ′), 13.4 (δ′); for g series (CH_2CHMe_2) 46.7 (α′), 28.1 (β′) [but 29.0 for γ-monoamide (**21g**)], 19.9 (γ′); for h series ($CHMeCH_2CH_3$), 46.5 (α′), 29.3 (β′), 10.2 (γ′), 20.0 (α′-Me); for i series (CMe_3) 51.1 (α′), 28.5 (β′); for j series (CH_2Ph) 43.3 (CH_2), 137.9 (1′), 127.4 (2′), 128.4 (3′), 127.2 (4′); for k series (C_6H_{11}), 48.2 (1′) [but 47.6 for non-acylated compounds (**12k**) and (**22k**)], 32.7 (2′), 24.5 (3′), 25.3 (4′); for l series ($-CH_2CH_2CH_2CH_2CH_2-$), 24.2, 25.4, and 26.0 (γ and *syn*/*anti* β), 43.1 and 46.4 or 42.6 and 46.2 [α of α-monoamide (**11l**) or of γ-monoamides (**21l**)]; and for m series ($=CH_2CH_2OCH_2CH_2-$), 42.2 and 45.7 [α of α-monoamide (**11m**)] or 41.8 and 45.6 (α of γ-monoamides), 66.4 and 66.6 [β of α-monoamide (**11m**)] or 66.1 and 66.3 (β of γ-monoamides). For the significance of italicized values, see the text. [b] δ_C 28.2 for α-sec-butylamide (**11h**). [c] δ_C 33.4 for γ-t-butylamide (**21i**). [d] δ_C 171.9 for α-methylamide (**11b**), 170.0 for α-cyclohexylamide (**11k**). [e] δ_C 172.4 for γ-methylamide (**21b**), 170.7 for γ-cyclohexylamide (**21k**).

* Signals within a vertical column may be interchanged.

methane chemical ionization, again showing characteristic C^{α}–CO cleavage. Thus *all* the α-monoamides gave rise to ion *b* of *m*/*z* 236 and (*b* − CO_2) of *m*/*z* 192, while *all* the γ-monoamides produced the characteristic ion *c*. Likewise ion *d* was found in the charge-exchange spectra of α- but not γ-monoamides. Further aspects of the mass spectra will be discussed in a later section.

The ^{13}C n.m.r. data for the same set of 26 monoamides (**11**) and (**21**) in deuteriochloroform solutions are summarized in Table 3. Also included are data for some non-acylated analogues (**12**) and (**22**). Chemical shift values which may be used to distinguish between α-amide γ-t-butyl esters (**11**) and γ-amide α-t-butyl esters (**21**) are shown in italics. First, the t-butoxy quaternary carbon of compounds of the α-amide-γ-ester series (**11**) resonates at 80.6 ± 0.2 p.p.m., while that of compounds of the γ-amide-γ-ester series (**21**) resonates at 81.8 ± 0.4 p.p.m. On comparing individual members of a given pair [*e.g.* α-amide (**11b**) *vs.* γ-amide (**21b**)], this carbon in an α-amide-γ-ester is seen to be 1.0—1.7 p.p.m. upfield of its counterpart in the γ-amide-α-ester. This shift difference is possibly steric in origin, as the apparent complementary shielding of the side-chain β-carbon (which is δ to the t-butoxy quaternary carbon) is observed. Thus the β-carbon resonance for an α-amide-γ-ester (**11**) is about 0.5 p.p.m. upfield of the same resonance for the corresponding γ-amide-α-ester (**21**).

For *NN*-disubstituted amides, a striking difference in chemical shift of the α-carbon (methines) is observed when the α-amide-γ-esters (**11l**) and (**11m**) (*ca.* 49.6 p.p.m.) are compared with the γ-amide-α-esters (**21l**) and (**21m**) (54.1 p.p.m.) (italicized in Table 3). The 4.5 p.p.m. shielding of this carbon in the former disubstituted amides compared with that in the monosubstituted amides (**21b—k**) is likely to be due to the γ-effect of the *syn* carbon on the amide nitrogen (see Figure, a). The corresponding shielding of the γ-methylene carbon in the latter disubstituted amides (**21l**) and (**21m**) (see Figure, b) is also observed (see italicized shifts in Table 3).

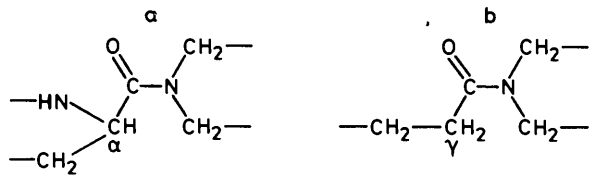


Figure.

Mass Spectra.—Reference has been made to the respective characteristic ions given by the α- and γ-monoamides (**11**) and (**21**) of t-butyl *N*-benzyloxycarbonyl-L-glutamate. Here we comment on other features of the spectra. With methane chemical ionization (Table 1), both α-and γ-monoamides yielded ions *e* and *f* derived formally from ion *a* ($MH^+ - C_4H_8$) by loss of CO_2 and $PhCH_2OH$ respectively. Four other sets of ions were given by members of both series. Ion *g* is the dehydrated analogue of ion *f*, while ion *h* corresponds to the loss of $PhCH_2OH$ from MH^+. Ion *i* is formally the MH^+ ion of the *N*-deprotected species (**12**/**22**) and gave rise to ion *j* on loss of C_4H_8. With helium charge-exchange (Table 2), cleavages of the benzyloxycarbonyl group become important, yielding the $PhCH_2^+$ ion (*m*/*z* 91) as the base peak. Ion *b* from the α-monoamides (**11**) and ion *c* from the γ-monoamides (**21**) were accompanied by corresponding ions 90 a.m.u. lower. Members of the α- and γ-monoamide series yielded, in addition to ion *f*, an ion *k* corresponding to the loss of $PhCH_2OCO$ from the (*M* −

Table 4. Mass spectra[a] of p-$CF_3CON(Me)C_6H_4CONHCH(COR^{\alpha})CH_2CH_2COR^{\gamma}$ (R^{α}/R^{γ} = NRR′/$OCMe_3$)

Compound	MH^+[b]	a[b]	$a - H_2O$ or $M - C_4H_9O$	MH^+ − CONRR′	b	c	a − NHRR′ or equiv.	a − HF	a − HF − NRR′	$CF_3CONMe\text{-}C_6H_4\overset{+}{C}O$	$CF_3\overset{+}{C}NH\text{-}(Me)C_6H_4CONH_2$	$\overset{+}{C}ONHCH(CONRR')\text{-}CH_2CH_2CO_2H$	$H_2NCH(CONRR')\text{-}CH_2CH_2\overset{+}{C}O$
(a) α-*Monoamides*													
(i) Methane chemical ionisation													
(13b)	*446**	*390*	372		331		*359*		339	230	247		
(13c)	*460**	*404*	386	388	331		359	384	339	*230*	247	201[d]	*157*
(13d)	*474**	*418*	400	388	*331*		359[e]	*398*	339	*230*	247	*215*	171
(13e)	*474**	*418*	400	388	331[d]		*359*[e]	*398*	339	230		215	
(13f)	*488**	*432*	*414*[d]	388	331		*e*	412			247	*229*	*185*
(13g)	*488**	*432*	414	388	331		359[e]	412	339				
(13h)	*488**	*432*	414	388	331[d]		359[e]	412	339	230		229	
(13i)	*488**	*432*	414	388	331[d]		359	412	339	230	247	229	
(13j)	*522**	*466*		*388*	*331*		359[e]	*446*	339	*230*	247	*263*	
(13k)	*514**	*458*	440	*388*	331[d]		*e*	*438*	339	*230*	247	*255*	211
(ii) Helium charge-exchange													
(13b)		389[c]	372	388	*331*					*230**	247		
(13c)		403[c]	386	388	*331*					*230**	247		
(13d)			*400*	388	*331*		359			*230**	247		
(13e)			400	*388*	331[d]					*230**	247		
(13f)				388	331					*230**			
(13g)			414	388	*331*[d]		359			*230**	247	229	
(13h)			414	388	*331*[d]		359			*230**	247		
(13i)			414	*388*	*331*[d]		359			*230**	247	*229*	
(13k)			440	388	331[d]					*230**			
(b) γ-*Monoamides*													
(i) Methane chemical ionisation													
(23k)	*514**	*458*				*412*		*438*		230	*247*		
(23l)	*500**	*444*				398		424		230			
(23m)	*502**	*446*				400		*426*		230			
(ii) Helium charge-exchange													
(23k)						412				*230**			
(23l)						398				*230**			
(23m)						400				*230**			

[a] See footnote *a* of Table 1. [b] The corresponding C_2H_5 adduct ions of 28 a.m.u. higher (and for M also the C_3H_5 adduct) were also observed. [c] ($M - C_4H_8$) ion. [d] Accompanied by an ion 1 a.m.u. higher (similar abundance). [e] The MH − NHRR′ ion (m/z 415) observed (abundance *ca.* 2%).

Table 5. Mass spectra[a] of *p*-MeNHC$_6$H$_4$CONHCH(COR$^\alpha$)CH$_2$CH$_2$COR$^\alpha$ (R$^\alpha$/R$^\gamma$ = NRR′/OCMe$_3$)

Compound	MH^+[b]/$M^{+\cdot}$	a[b]/M − C_4H_8	a − H_2O or M − C_4H_9O	b	c	MH^+ − NHRR′	a − NHRR′ *or equiv.*	$MeNHC_6H_4\overset{+}{C}O$	$MeN\overset{+}{H}_2C_6H_4$-CONH$_2$	[RR′NCOCH$_2$-CH$_2$CH$_2$CO$_2$H·H]$^+$	$\overset{+}{C}$ONHCH(COR$^\alpha$)CH$_2$CH$_2$COR$^\alpha$ (R$^\alpha$/R$^\gamma$ = NRR′/OH)	$H_2NCH(CO\overset{+}{N}RR')$-CH$_2CH_2$CO
(a) α-*Monoamides*												
(i) Chemical ionisation												
(14a)	*336*	*280*				*319*	*263*	*134**	*151*			
(14b)	*350*	*294*	*276*			*319*	*263*	*134**	*151*		187	143
(14c)	*364*	*308*	*290*	235		*319*	*263*	*134**	151		201	157
(14d)	*378*	*322*	304	235		*319*	*263*	*134**	151	*174*	*215*	171
(14e)	*378**	*322*		235		*319*	*263*	*134*			215	171
(14f)	*392*	*336*		*235*		*319*	*263*	*134**			*229*	185
(14g)	*392*	*336*	318	*235*		*319*[b]	*263*[b]	*134**				185
(14h)	*392**	*336*	318	*235*		*319*	*263*[b]	*134*	151		*229*	185
(14i)	*392*	*336*	318	*235*		*319*	*263**,[b]	*134*	*151*	*188*	*229*	185
(14j)	*426*	*370*	352	235		*319*	*263*[d]	*134**	*151*	222	*263*[d]	
(14k)	*418*	*362*	344	235		*319*	*263*	*134**	*151*	*214*	*255*	211
(*ii*) Helium charge-exchange												
(14b)	349			235			263	*134**				
(14c)	363			235[e]			263	*134**				
(14d)	377			*235*[e]			263	*134**				
(14e)	377			235[e]			263	*134**				
(14f)	391			235[e]			263	*134**				
(14g)	391			235[e]			263	*134**				
(14h)	391			235[e]			263	*134**				
(14i)	391			*235*[e]			263	*134**				
(14k)	417			235			263	*134**				
(b) γ-*Monoamides*												
(i) Methane chemical ionisation												
(24a)	*336*	*280**	262					*134*	151			
(24k)	*418*	*362*						*134*	*151**	*214*	255	
(24l)	*404**	*348*	330		302			*134*	*151*	200	*241*	
(24m)	*406*	*350**	332		304			*134*	*151*	202	*243*	
(ii) Helium charge-exchange												
(23k)[c]	417	361	344					*134**	151			
(23l)[c]	403	347	330		302			*134**	151			
(23m)[c]	405	349	332		304			*134**	*151*			

[a] See footnote *a* of Table 1. [b] See corresponding footnote for Table 4. [c] Also given by compounds **(24k)**, **(24l)**, and **(24m)** respectively are ions of *m/z* 227, 213, or 215 (M − C_4H_8 − 134); 182, 168, or 170 (c − 134); and 210, 196, or 198 (a − H_2O − 134); abundances 1 − 10%. [d] There are two origins of the *m/z* 263 ion. [e] Accompanied by *m/z* 292 ion (MH − CONRR′) of *ca.* 1/5th abundance.

Table 6. ^{13}C Chemical shifts of *p*-methylaminobenzoyl-L-glutamic acid monoamide t-butyl esters[a]

	α-Monoamides				γ-Monoamides					
Compounds	(13b—k)	(14b—k)	(18d)	(19a)	(23d), (23k)	(23l), (23m)	(24d), (24k)	(24l), (24m)	(28a)	(29a)
NCH_3	39.1[g]	29.9[j]	39.0	38.9	39.1	39.1	29.9	29.8	39.2	38.9
$OC(CH_3)_3$	27.7	27.8[j]	27.8	27.8	27.8	27.7	27.7	27.7	27.9	27.7
$^{\beta}CH_2CH_2CO$	27.2—27.5[d]	27.3—27.6[d]	27.3	26.9	27.1/27.4	26.2	29.0/28.6	27.2	28.2	27.7
$CH_2{}^{\alpha}CH_2CO$	31.6	31.7[j]	31.7	31.7	32.4/32.8	29.4/29.1	32.7	29.4/29.0	32.0	31.8
$NH{}^{\alpha}CHCO$	53.2[g]	52.6—53.0	52.9	52.9	53.4	53.8/53.4	52.7	53.0/52.7	52.9	52.8
$OC(CH_3)_3$	80.5—80.9[e]	80.2—80.5	80.6	80.7	82.1	81.6/81.9	82.1/81.7	81.4/81.6	82.3	82.0
NC_6H_4CO (1′)	133.9	121.0—121.3[j]	121.8	121.0	134.1	134.1	121.2	121.5/121.1	122.0	121.2
(2′)	128.5	128.7	128.8	128.9	128.5	128.4	128.6	128.5	128.9	128.7
(3′)	127.0	111.0[j]	111.1	111.2	127.1	127.0	111.0	111.0	111.4	111.0
(4′)	143.1—143.4	151.9	150.6	150.9	*h*	143.1	152.0	151.9	150.7	150.9
CF_3CO	116.0[b]				*h*	116.0				
CF_3CO	156.3[c]				*h*	156.3				
C_6H_4CO	165.9[g]	167.0—167.4	167.0	167.3	165.8	165.4	167.3	166.8	167.3	167.2
$^{\gamma}CH_2C(O)O$	172.6—172.9[e]	172.6—173.0[i]	172.9	173.0						
$^{\alpha}CHC(O)O$					170.7	170.6/171.0*	171.3*	171.4*/171.2*	171.5	171.4
$^{\alpha}CHC(O)NRR'$	170.2—171.6[f]	170.5—171.6[f]	171.5	174.8						
$^{\gamma}CH_2C(O)NRR'$					172.4/171.4	170.6/170.0*	172.3/171.2*	170.3*/170.8*	175.3 131.9	175.4 144.7
Pyrazine CH(3)			131.7	144.8						
Pyrazine C(5)			149.2**	155.6					149.2**	155.7
(2)			144.7**	143.0					145.5**	143.0
(6)			113.9*	115.2*					114.0*	115.1*
Pyrazine 6-*C*N			112.9*	112.0*					113.2*	119.9*
Pyrazine 2-CH_2NMe			55.2	54.9					55.1	54.7

[a] δ_C In p.p.m. downfield from $SiMe_4$ in $CDCl_3$ (to ±0.1 p.p.m.), $\delta_C(CDCl_3)$ 76.9 p.p.m. For chemical shifts of amide *N*-alkyl carbons, see footnote *a* of Table 3. [b] Quartet with J_{CF} 288.9 Hz. [c] Quartet with J_{CCF} 35.3 Hz. [d] δ_C 27.8—28.0 p.p.m. for α-monoamides with branching at C-1′ (series e, h, i) [except for compound (**13h**), δ_C 27.6 p.p.m.]. [e] For α-propylamide (**13d**), δ_C 172.2 and 80.2 p.p.m. for $C(O)OCMe_3$. [f] δ_C 171.8 and 172.3 p.p.m. for α-methylamides (**13b**) and (**14b**) respectively; δ_C 170.0 p.p.m. for α-cyclohexylamide (**13k**). [g] For α-t-butylamide (**13l**), δ_C 39.6 for NCH_3, 53.4 for α′-CH, and 165.6 for C_6H_4CO. [h] Not observed (small sample). [i] δ_C 172.0 p.p.m. for α-butylamide (**14f**). [j] About 0.2 p.p.m. to higher field for the α-propylamide (**14d**).

*,** Signals within a vertical column may be interchanged.

C_4H_8) ion. The γ-monoamides yielded a characteristic *m/z* 174 ion, and a set of ions which may be derived from ion *c* by the loss of $PhCH_2OCONH_2$.

Mass matching under electron-impact conditions (which yielded spectra similar to those from helium charge-exchange) on selected examples of α-monoamides [(**11i**) and (**11m**)] and γ-monoamides [(**21i**) and (**21m**)] indicates the correctness of the above discussed structural assignments to ions *b*, *b* − CO_2, *b* − PhCH, and *d* derived from the α-monoamides; ions *M* − C_4H_8, *c*, and *k* derived from the γ-monoamides; and the *m/z* 91 ion from both series.

The *p*-acyl(methyl)amino derivatives (**13**) and (**23**) (Table 4), and methylamino derivatives (**14**) and (**24**) (Table 5), also showed chemical ionization and charge-exchange spectra diagnostic of α- *vs.* γ-monoamides. Thus characteristic C^{α}–CO cleavage of ($MH^+ - C_4H_8$) or ($M - C_4H_8$) gave ion *b* from the α-monoamides (**13**) (*m/z* 331, Table 4) and (**14**) (*m/z* 235, Table 5); and sets of ion *c* from the γ-monoamides (**23**) (Table 4) and (**24**) (Table 5). For the α-monoamides (**13**) and (**14**), cleavage of *a* and/or MH^+ at CO–NRR′ were also observed giving rise to, for compounds (**13**), ions at *m/z* 359 (Table 4), and for compounds (**14**), ions at *m/z* 263 (for chemical ionization, also at 319) (Table 5). Both α- and γ-monoamides gave rise to ions due to cleavage on either side of the *p*-aminobenzoyl carbonyl, and to breaking of the NH–C^{α} bond. Those with charge retention on the *p*-aminobenzoyl moiety are ions *m/z* 230 and 247 from compounds (**13**) and (**23**) (Table 4), and their analogues, *m/z* 134 and 151, from compounds (**14**) and (**24**) (Table 5). Charge retention on the glutamyl residue resulted in series of ions which may be formulated as $^+CONHCH(COR^{\alpha})CH_2CH_2COR^{\gamma}$, $[R^{\alpha}COCH_2CH_2CH_2COR^{\gamma}]H^+$ (R^{α}/R^{γ} = OH/NRR′), and $H_2NCH(CONRR')CH_2CH_2{}^+CO$ (Tables 4 and 5, right-hand columns).

^{13}C *N.m.r. spectra.*—Data for various monoamides of t-butyl *p*-methylaminobenzoylglutamate are summarized in Table 6. The difference in t-butoxy carbonyl carbon resonances characteristic of α- *vs.* γ-monoamides is again observed (*ca.* 80.5 *vs. ca.* 82 p.p.m.), as was discussed earlier in relation to the benzyloxycarbonyl derivatives (**11**) and (**21**). For both benzyloxycarbonyl (Table 3) and *p*-methylaminobenzoyl derivatives (Table 6), series of α-monoamide γ-t-butyl esters (except *NN*-disubstituted amides) show consistency in γ-methylene (31.5 ± 0.2 p.p.m.) and γ-carbonyl (172.7 ± 0.3 p.p.m.) shieldings. In the case of benzyloxycarbonyl derivatives (for which an 'homologous' series is available) (Table 3), the γ-monoamide α-t-butyl esters (**21a**—**m**) show a parallel regularity in α-methine (54.0 ± 0.1 p.p.m.) and α-carbonyl (170.9 ± 0.1 p.p.m.) shieldings. For this series of γ-monoamides, the γ-effect of the *syn N*′-alkyl group on the γ-methylene signal has been mentioned earlier. The same effect is seen for the *p*-methylaminobenzoyl derivatives (Table 6) when carbon shifts of the γ-

methylenes of the disubstituted amides (**23l, m**), (**24l, m**) are compared with those of the monosubstituted amides (**23d, k**), (**24d, k**) (*ca.* 29.3 *vs. ca.* 32.7 p.p.m.). The steric effect of *N'*-alkyl groups is also observed for α-monoamides. Thus, those with branching at C-1 (t-butyl, sec-butyl, cyclohexyl, and isopropyl) show somewhat more deshielded β-carbons [see Table 3, column (**11i**) and footnote *b*; Table 6, footnote *d*], while α-t-butylamides tend to have in addition low-field α-methines [Table 3, column (**11i**); Table 6, footnote *g*] (δ effect). The above branched chains also cause some shielding of the α-carbonyl which is γ to it; the reverse effect is observed for α-methylamides (Table 3, footnote *d*; Table 6, footnote *f*). For unsubstituted primary amides, the $CONH_2$ signal occurs consistently some 3 p.p.m. downfield of the corresponding monosubstituted amide carbonyl signal [Table 3, compounds (**11a**), (**21a**); Table 6, compounds (**13a**), (**28a**), (**29a**)].

Included in Table 3 are data for some *N*-unprotected derivatives, one of which [the γ-monoamide (**22m**)] is in the protonated form. Comparison of the free amines (**12k**), (**22d**), and (**22k**) with their *N*-benzyloxycarbonyl analogues shows that the shielding effect of benzyloxycarbonylation is about 2 p.p.m. at the β-carbon and 3 p.p.m. at the α-carbonyl. The upfield effect of protonation is, as expected,[18] bigger at the β-carbon (4 p.p.m.) than at the α (1 p.p.m.), and is not discernible at γ.

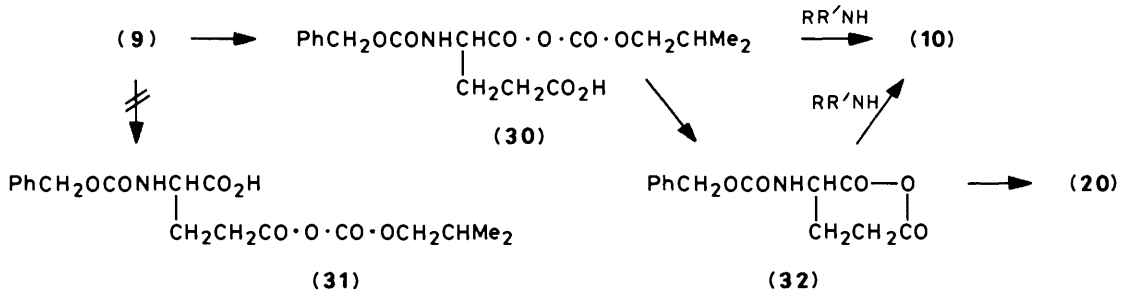
γ/α Ratio in the Mixed-anhydride Coupling of Amines to N-*Benzyloxycarbonyl*-L-*glutamic Acid.*—A striking difference in the ratio of α- to γ-monoamides was observed when comparing the coupling of ammonia and primary and secondary amines, by the mixed anhydride method, to *N*-benzyloxycarbonyl-L-glutamic acid (**9**). Under identical coupling conditions utilizing one equivalent of isobutyl chloroformate (Experimental section), and after conversion into t-butyl esters, the isolated yields of α- and γ-monoamide t-butyl esters (**11**) and (**21**) were such that the γ/α ratio varied from 0.04 to 3.5 (Table 7). Reaction with ammonia gas gave only the α-monoamide as was observed by Ressler.[8] Ressler postulated that treatment of the triethylamine salt of *N*-benzyloxycarbonyl-L-glutamic acid (**9**) in tetrahydrofuran (THF) at below −20 °C with one equivalent of the chloroformate preferentially activates the α-carboxyl group to give the α-mixed anhydride (**30**), which on subsequent addition of ammonia yields solely the α-monoamide (**10**) (Scheme 3).[8] We observed similar results with methylamine (γ/α ratio 0.04). The minimal degree of γ-monoamide formation in the above cases indicates that the corresponding γ-mixed anhydride (**31**), if present at all, was at a much lower level. However, it appears that with less reactive amines, cyclization to the cyclic anhydride (**32**) could take place, especially when the temperature was allowed to rise above −20 °C (see Experimental section). This postulated intermediate would be subject to attack at both carbonyl groups, resulting in substantial amounts of the γ-monoamide (**20**) being formed. It had been reported that starting with *N*-benzyloxycarbonyl-L-glutamic anydride (**32**), varying amounts of α- and γ-products were obtained on reaction with amines and alcohols.[21].

We attempted to relate the observed γ/α ratio to some property of the attacking amines, but restricting the discussion to the 10 primary amines studied (see lines 2—11 of Table 7). The situation for secondary amines may be complicated by the formation of pyroglutamic acid intermediates.[22] The γ/α ratio for reaction with primary amines RNH_2 bears no obvious relationship to the steric parameter the Hancock constant E_s^c for R groups,* nor to the Taft electronic parameter σ*.†

Table 7.

Amines RR'NH	% Yield[a] of α-amide (**11**)	% Yield[a] of γ-amide (**21**)	γ/α Ratio	Connectivity $^1\chi$ of RR'NH[b]
NH_3	53[c]		0.02	
Primary amines (R' = H)				
CH_3NH_2	27	1	0.04	1.00
$CH_3CH_2NH_2$	29	3	0.10	1.41
$(CH_3)_2CHNH_2$	30	2	0.07	1.73
$CH_3CH_2CH_2NH_2$	32	15	0.47	1.91
$(CH_3)_3CNH_2$	10[d]	3[d]	*ca.* 0.3[d]	2.00
$(CH_3)_2CHCH_2NH_2$	20	5	0.25	2.27
$CH_3CH_2CH(CH_3)NH_2$	33	9	0.27	2.27
$CH_3CH_2CH_2CH_2NH_2$	16	10	0.63	2.41
cyclo-$C_6H_{11}NH_2$	37	24	0.64	2.89[e]
$PhCH_2NH_2$	17	19	1.1	3.43[e,f]
Secondary amines (R' = alkyl)				
Morpholine	5	14	2.8	
Piperidine	8	28	3.5	

[a] Overall isolated yields of α- and γ-monoamide t-butyl esters (**11**) and (**21**) from mixed anhydride coupling of RR'NH to *N*-benzyloxycarbonyl-L-glutamic acid, followed by esterification. [b] First-order molecular connectivity.[19,20a] [c] Before esterification, yield was 71%; Ressler[8] reported 50%, and also no sign of the γ-amide. [d] Both products were non-crystalline. [e] Modifying term of −0.50 used for monocyclic system.[20c] [f] Computed according to Randić.[19, 20d]



Scheme 3.

* For primary amines, values are (in the order listed in Table 7) 0.00, −0.38, −1.08, −0.67, −2.46, −1.24, −1.74, −0.70, −1.40, −0.69 [as calculated from the E_s values of Taft (C. Takayama, T. Fujita, and M. Nakajima, *J. Org. Chem.*, 1979, 44, 2871)].

† For primary amines in order listed: 0.00, −0.10, −0.19, −0.10, −0.30, −0.13, −0.21, −0.13, −0.15, +0.22; data of R. W. Taft in 'Steric Effects in Organic Chemistry,' ed. M. S. Newman, Wiley, New York, 1956, pp. 556—675.

Likewise the observed γ/α ratio is not a function of the pK_a values of the amines (10.4—10.7, except for benzylamine with a value of 9.4),[23] a composite of electronic and steric factors. Nevertheless a relationship to the first-order connectivity $^1\chi$ may be noted. The index $^1\chi$ is a topological branching index[19,20a] which in the case of primary amines has been correlated with molar properties such as boiling point[20b] and n-octanol–water partition coefficient.[24] Table 7 shows that the order of increasing $^1\chi$ for the 10 primary amines listed generally parallels the order of increasing γ/α ratio for 'attack' by these amines.* However, as the γ/α ratio is the outcome of consecutive and parallel reactions, this observation must be considered as a qualitative one.

Experimental

^{1}H and ^{13}C N.m.r. data are given in Tables 8 (^{1}H), 3, and 6(^{13}C), and were collected using JEOL FX-90Q (^{1}H and ^{13}C) and Varian CFT-20 (^{13}C) Fourier-transform spectrometers, and a Varian HX-100 continuous-wave spectrometer (^{1}H). U.v. data of methotrexate analogues (**15**), (**16**), (**25**), and (**26**) are given in Table 9. Most chemical ionization and all charge-exchange mass spectral data are collated in Tables 1, 2, 4, and 5, and were measured using a Finnigan 3200E quadrapole mass spectrometer. Some 70 eV electron-impact data are given in this section [compounds (**14a**), (**24a**), (**13j**), and (**13h**)]. Molecular weight determinations by e.i.m.s or by c.i.m.s refer to (respectively) electron-impact or chemical ionization mass spectrometry, and were performed on AEI MS-902 spectrometers. Accurate mass measurements under hydrogen gas chemical ionization conditions (Scientific Research Instruments CIS2 CI/EI source) were made at a resolution of 5 000 from a VU chart recorder using a manual peak-centre-determination method.[25] Where descriptions of two or more isomers follow one another, the calculated elemental analysis values may be listed once only. M.p.s. were uncorrected, and light petroleum refers to the fraction of b.p. 40—60 °C. T.l.c. refers to separation over silica gel plates; and chromatography over silica gel refers to separation either under several Torr pressure on a short column of t.l.c.-grade silica gel, or under gravity on a long column of 100—235 mesh silica gel, usually with elution using chloroform, or chloroform–methanol mixtures. Samples characterized by elemental analysis or by mass spectrometric mass matching were checked for purity using high-pressure liquid chromatography (h.p.l.c.) and/or t.l.c. The former tests were performed on a system consisting of an Altex model 100 pump, a model 153 u.v. (254 nm) detector, and a Rheodyne model 7120 injection valve. The reverse-phase analytical column used (0.46 × 25 cm) was packed with Merck RP-8 (10 μm) material, and elution was by water–methanol mixtures. Amino acid derivatives were purchased from Bachem Inc., Torrance, California. Ether is diethyl ether.

Preparation of N-*Benzyloxycarbonyl-*L-*glutamic Acid Monoamides* (**10**) *and* (**20**).—The following preparation of the monopropylamides of *N*-benzyloxycarbonyl-L-glutamic acid illustrates the general procedure. A solution of *N*-benzyloxycarbonyl-L-glutamic acid (**9**) (10.0 g, 35.5 mmol) and triethylamine (10.0 ml, 72.2 mmol) in dry, freshly distilled THF (60 ml) was cooled to below −20 °C with protection from moisture. Isobutyl chloroformate (4.7 ml, 43.9 mmol) was added dropwise to the stirred mixture during 30 min, the temperature being maintained at −20 °C, and yielded a white precipitate. After the mixture had been kept for 30 min at the same temperature, propylamine (8.8 ml, 107 mmol) was added dropwise to the stirred mixture during 30 min. The solution was then allowed to attain room temperature (*ca.* 1 h). Ether (200 ml) was then added and an oily precipitate was formed. After the supernatant was removed, the residue was washed with ether (50 ml) and the residue, after evaporation of the remaining organic solvent, was dissolved in water (150 ml). The aqueous solution was filtered, cooled, and acidified with 4M hydrochloric acid to pH 4, to give a precipitate which was collected, washed (water), and dried under reduced pressure. The resulting mixture consisted of *N*-benzyloxycarbonyl-L-glutamic acid α- and γ-propylamide (**10d**) and (**20d**) (10.2 g, 90%). These and other monoamides of *N*-benzyloxycarbonyl-L-glutamic acid prepared by the same procedure (but sometimes worked up by addition of water, and extraction of the acidified mixture by ethyl acetate) were converted without further purification into the respective t-butyl esters (**11**) and (**21**) for separation into the α- and γ-series, as described further below. However, for some *N*-benzyloxycarbonyl-L-glutamic acid monoamides, separation was carried out directly prior to formation of the t-butyl esters. An example is described immediately below.

A mixture of monobenzylamides prepared from benzylamine and *N*-benzyloxycarbonyl-L-glutamic acid (**9**) (4.0 g) was crystallized from chloroform–ether to give needles of N-*benzyloxycarbonyl-*L-*glutam-α-benzylamide* (N′-*benzyl*-N-*benzyloxycarbonyl-*L-*isoglutamine*) (**10j**) (1.00 g), m.p. 158—159 °C (Found: C, 64.75; H, 6.1; N, 7.35. $C_{20}H_{22}N_2O_5$ requires C, 64.85; H, 6.0; N, 7.55%); and needles of *N*-benzyloxycarbonyl-L-glutam-γ-benzylamide (*N*′-benzyl-*N*-benzyloxycarbonyl-L-glutamine)[22] (**20j**) (0.85 g) (Found: C, 64.55; H, 6.0; N, 7.45%).

N-Benzyloxycarbonyl-L-isoglutamine (**10a**), an α-amide, was the major monoamide obtained when the mixed anhydride of *N*-benzyloxycarbonyl-L-glutamic acid was coupled as described previously with dry ammonia gas. The crystalline solid obtained was recrystallized from water to give needles (68% yield) of the α-amide (**10a**), m.p. 169—171 °C (lit.,[14] 171—173 °C).

Preparation of t-Butyl Esters of N-*Benzyloxycarbonyl-*L-*glutamic Acid α-and γ-Monoamides.*—The following illustrates the general procedure.[26] A mixture of *N*-benzyloxycarbonyl-L-glutamic acid α- and γ-propylamide (**10d**) and (**20d**) [prepared from *N*-benzyloxycarbonyl-L-glutamic acid (35.5 mmol)] was suspended in t-butyl acetate (500 ml) containing 70% w/v perchloric acid (2.7 ml, 32 mmol). After being shaken for 4 h the mixture became homogeneous; t.l.c. indicated completion of the reaction. After addition of ethyl acetate (500 ml), the organic solution was washed successively with water (2 × 200 ml), saturated aqueous sodium hydrogen carbonate (until CO_2 evolution ceased), and finally saturated aqueous sodium chloride (200 ml). The organic phase was dried (sodium sulphate) and evaporated. Chromatography of the residue over silica gel gave, in order of elution, N-*benzyloxycarbonyl-*L-*glutam-α-propylamide t-butyl ester* (**11d**) (N-*benzyloxycarbonyl*-N′-*propyl-*L-*isoglutamine t-butyl ester*) (8.6 g) as needles from ether–light petroleum, m.p. 84.5 °C (Found: C, 63.6; H, 7.9; N, 7.35. $C_{20}H_{30}N_2O_5$ requires C, 63.45; H, 8.0; N, 7.4%), and N-*benzyloxycarbonyl-*L-*glutam-γ-propylamide t-butyl ester* (N-*benzyloxycarbonyl*-N′-*propyl-*L-*glutamine t-butyl ester*) (**21d**) (4.2 g) as needles from ether–light petroleum, m.p. 97 °C (Found: C, 63.55; H, 8.1; N, 7.4%).

In a similar manner, the following monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate were prepared.

α- and γ-*Methylamide* (**11b**) and (**21b**), m.p. 97—98 °C and 106—107 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 61.95; H, 7.25; N, 8.1. Found for γ-amide: C, 61.85; H, 7.45; N, 7.85. $C_{18}H_{26}N_2O_5$ requires C, 61.7; H, 7.5; N, 8.0%).

α- and γ-*Ethylamide* (**11c**) and (**21c**), m.p. 86—88 °C and 72—

* γ/α Ratio = 0.42 $^1\chi$ − 0.52 (sets of data = 10, standard deviation = 0.15, correlation coefficient = 0.90).

73 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 62.55; H, 7.7; N, 7.9. Found for γ-amide: C, 62.4; H, 7.7; N, 7.4. $C_{19}H_{28}N_2O_5$ requires C, 62.6; H, 7.75; N, 7.7%).

α- and γ-*Isopropylamide* (**11e**) and (**21e**), m.p. 86—87 °C and 113—114 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 63.3; H, 7.7; N, 7.6. Found for γ-amide: C, 63.7; H, 7.95; N, 7.4. $C_{20}H_{30}N_2O_5$ requires C, 63.45; H, 8.0; N, 7.4%).

α- and γ-*Butylamide* (**11f**) and (**21f**), m.p. 70—70.5 °C and 86—87 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 64.5; H, 8.35; N, 7.05. Found for γ-amide: C, 64.05; H, 8.1; N, 7.55. $C_{21}H_{32}N_2O_5$ requires C, 64.25; H, 8.2; N, 7.15%).

α- and γ-*Isobutylamide* (**11g**) and (**21g**), m.p. 81—82 °C and 106—107 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 64.05; H, 8.1; N, 7.1. Found for γ-amide: C, 64.5; H, 8.3; N, 7.15%).

α- and γ-*sec-Butylamide* (**11h**) and (**21h**), m.p. 81—82 °C and 103—104 °C respectively (needles from ether–light petroleum) (Found for α-amide: C, 64.15; H, 8.2; N, 7.2. Found for γ-amide: C, 64.6; H, 8.2; N, 7.15%).

α- and γ-*t-Butylamide* (**11i**) and (**21i**) were obtained as low melting solids after silica chromatography followed by preparative h.p.l.c. (Found: M^+ by e.i.m.s., 392.233 and 392.232 respectively. $C_{21}H_{32}N_2O_5$ requires M, 392.231).

α- and γ-*Cyclohexylamide* (**11k**) and (**21k**), m.p. 116—117 °C and 151—153 °C respectively (needles from ethyl acetate) (Found for α-amide: C, 65.85; H, 8.3; N, 6.6. Found for γ-amide: C, 66.0; H, 8.5; N, 6.45. $C_{23}H_{34}N_2O_5$ requires C, 66.0; H, 8.2; N, 6.7%).

α- and γ-*Piperidide* (**11l**) and (**21l**); the former was obtained as a gum (Found: M^+ by e.i.m.s., 404.237. $C_{22}H_{32}N_2O_5$ requires M, 404.239), and the latter as needles (from ether–light petroleum), m.p. 88—89 °C (Found: C, 65.3; H, 8.5; N, 6.8. $C_{22}H_{32}N_2O_5$ requires C, 65.3; H, 7.95; N, 6.95%).

α- and γ-*Morpholide* (**11m**) and (**21m**) were obtained as gums after repeated silica chromatography (Found: $M - C_4H_8$ by e.i.m.s., 350.150 and 350.148 respectively. $C_{17}H_{22}N_2O_6$ requires m/z 350.148).

Similar esterification[26] of individual pure samples of *N*-benzyloxycarbonyl-L-glutamic acid monoamides yielded the following monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate: α-benzylamide (**11j**) (70% yield), m.p. 89—91 °C (from ether–cyclohexane) (Found: C, 68.15; H, 7.2; N, 6.55. $C_{24}H_{30}N_2O_5$ requires C, 67.6; H, 7.1; N, 6.55%); γ-benzylamide (**21j**) (79% yield), m.p. 78—80 °C (needles from ether–cyclohexane) (Found: C, 67.25; H, 6.95; N, 6.35%); t-butyl *N*-benzyloxycarbonyl-L-isoglutamine (**11a**) (75% yield), m.p. 134—136 °C (lit.,[27] 132—133 °C) (Found: C, 60.4; H, 7.15; N, 8.3. Calc. for $C_{17}H_{24}N_2O_5$: C, 60.7; H, 7.2; N, 8.35%); t-butyl *N*-benzyloxycarbonyl-L-glutamine (**21a**) [75% yield from commercial *N*-benzyloxycarbonyl-L-glutamine (**20a**)], m.p. 93—94 °C (lit.,[9] 94—95 °C) (Found: C, 60.45; H, 7.15; N, 8.5%).

Hydrogenolysis of Monoamides of t-Butyl N-*Benzyloxycarbonyl-L-glutamate.*—*N*-Benzyloxycarbonyl-L-glutam-γ-cyclohexylamide (*N*-Benzyloxycarbonyl-*N'*-cyclohexyl-L-glutamine) t-butyl ester (**21k**) (0.73 g) in methanol (25 ml) was hydrogenated at atmospheric pressure in the presence of 10% palladium–charcoal catalyst (40 mg). When t.l.c. indicated the completion of the reaction, the filtered solution was evaporated (with addition of dioxane to aid complete removal of methanol) to yield L-glutam-γ-cyclohexylamide (*N'*-cyclohexyl-L-glutamine) t-butyl ester (**22k**) as a gum, m/z (CH_4 c.i.) 313 (5%, $M + C_2H_5$), 285 (25, MH^+), 257 (15, $M + C_2H_5 - C_4H_8$), 229 (100, $MH^+ - C_4H_8$), 183 (35, *c*), and 100 (20, $C_6H_{11}NH_3^+$). This was used immediately for the next reaction.

The same method (with methanol or ethyl acetate as solvent) was used to hydrogenate the various other α- and/or γ-monoamides of t-butyl *N*-benzyloxycarbonyl-L-glutamate described in the previous sub-section to yield α- and/or γ-monoamides of t-butyl L-glutamate which were used without purification as starting materials in the reactions described below. Of these only the α-cyclohexylamide (**12k**) was obtained crystalline, with m.p. 57—58 °C, m/z (CH_4 c.i.) 313 (2%, $M + C_2H_5$), 285 (5, MH^+), 257 (5, $M + C_2H_5 - C_4H_8$), 229 (100, $MH^+ - C_4H_8$), 211 (40, 229 − H_2O), 158 (25, $MH^+ - HCONHC_6H_{11}$), and 102 (30, *b*). One of the monoamides prepared, the L-glutam-γ-morpholide t-butyl ester (**22m**), was characterized as the *hydrochloride* (85% yield), m.p. 159—160 °C (from chloroform–ether) (Found: C, 50.5; H, 8.15; N, 9.0; Cl, 11.8. $C_{13}H_{24}N_2O_4$·HCl requires C, 50.55; H, 8.15; N, 9.50; Cl, 11.6%); m/z (CH_4 c.i.) 301 (10%, $M + C_2H_5$), 273 (45, MH^+), 245 (20, $M + C_2H_5 - C_4H_8$), 218 (25), 217 (100 $MH^+ - C_4H_8$), 216 (40), 200 (15, $MH^+ - OCMe_3$), and 171 (30, *c*); m/z (He charge-exchange) 273 (2%), 217 (6), 216 (4), 215 (2), 200 (2), 171 (8), 139 (10), 88 (100), and 86 (85).

Monoamides of t-Butyl N-[p-*Methyl(trifluoroacetyl)aminobenzoyl*]-L-*glutamate.*—(*a*) *p*-Methyl(trifluoroacetyl)aminobenzoic acid, m.p. 173—175 °C (6.25 g, 25.3 mmol) (prepared by reaction of *p*-methylaminobenzoic acid with trifluoroacetic anhydride and trifluoroacetic acid in dioxane) was dissolved in dry dioxane (120 ml). Triethylamine (1.2 ml, 8.4 mmol) was added to the stirred solution, followed by isobutyl chloroformate (3.48 ml, 25.3 mmol). T.l.c. indicated that the formation of the mixed anhydride (a white precipitate) was complete in 10 min. A solution of L-glutam-γ-cyclohexylamide (*N'*-cyclohexyl-L-glutamine) t-butyl ester (**22k**) (23.0 mmol) in dioxane (50 ml) was added. After 2 h, when t.l.c. indicated the complete consumption of the mixed anhydride, the solution was evaporated under reduced pressure. The residue was partitioned between saturated aqueous sodium chloride (25 ml) and ethyl acetate (200 ml). The organic phase was washed successively with saturated aqueous sodium hydrogen carbonate (2 × 15 ml) and saturated aqueous sodium chloride (15 ml), dried (sodium sulphate) and evaporated to give, after chromatography over silica, N-[p-*methyl(trifluoroacetyl)aminobenzoyl*]-L-*glutam-γ-cyclohexylamide* {N'-*cyclohexyl*-N-[p-*methyl*-(*trifluoroacetyl*)*aminobenzoyl*]-L-*glutamine*} *t-butyl ester* (**23k**) (4.0 g) as needles from ethyl acetate, m.p. 158—159 °C (Found: C, 58.3; H, 6.8; N, 8.05. $C_{25}H_{34}F_5N_3O_5$ requires C, 58.45; H, 6.65; N, 8.2%).

Starting with the appropriate α- or γ-monoamide of t-butyl L-glutamate and using the same coupling method (with ethyl acetate or dichloromethane work-up), the following monoamides of t-butyl *N*-[*p*-methyl(trifluoroacety)laminobenzoyl]-L-glutamate were obtained as gums after purification by chromatography over silica: α-propylamide (**13d**) (Found: MH^+ by H_2 c.i.m.s., 474.219. $C_{22}H_{31}F_3N_3O_5$ requires MH, 474.221); α-benzylamide (**13j**) (Found: M^+ by e.i.m.s., 521.214. $C_{26}H_{30}F_3N_3O_5$ requires M, 521.214). Also prepared were the α-amide (**13a**), the γ-amide (**23a**), and the α-cyclohexylamide (**13k**), all of which were characterized after removal of the *N*-trifluoroacetyl group (below). The α-benzylamide (**13j**) showed m/z (e.i.m.s) 521 (0.5%, $M^{+\cdot}$), 448 (2, $M - OC_4H_9$), 388 (7, $MH^+ - CONHCH_2C_6H_5$), 332 (14), 331 (40, *b*), 231 (16), 230 (100, $CF_3CONHMeC_6H_4CO^+$), and 110 (40).

(*b*) A solution of L-glutam-α-ethylamide (*N'*-ethyl-L-isoglutamine) t-butyl ester (**12c**) (11.0 mmol) in dry dioxane (30 ml) containing triethylamine (1.68 g, 12.1 mmol) was added dropwise to a stirred solution of *p*-methyl(trifluoroacetyl)aminobenzoyl chloride [obtained by refluxing the corresponding acid (2.71 g, 12.1 mmol) with thionyl chloride (30 ml) for 1 h followed by evaporation of excess of reagent] in dry dioxane (30

Table 8. ^{1}H N.m.r. chemical shifts and (*J*/Hz in parentheses)[a]

α-Monoamides γ-Monoamides	(**11a—m**)[b] (**21a—m**)[b]	(**12k**)[b] (**22d, k**)[b]	(**13b—k**)[b] (**23d, k, l, m**)[b]	(**14a**),[d] (**14b—k**)[b] (**24a, d, k, l, m**)[b]	(**15a—j**),[c] (**15k**),[e] (**25a, d**),[d] (**25l**),[c] (**25m**)[a]	(**15f**)[d] (**25k**)[d]
*Ph*CH$_2$	7.3—7.35					
ArC*H*$_2$	5.05—5.1				4.7—4.8	4.8
N*H*αCH	*ca.* 6d (8)		*ca.* 7.5d (7.5)	*ca.* 7.5d (7.5)	*ca.* 7.5d (7.5)	
αCCON*H*R	*ca.* 6.5m[f]	*ca.* 7m	*ca.* 7m[f]	*ca.* 7m[f]	*ca.* 6.5m[f]	
NαC*H*CO	4.1—4.3m[g] or 4.7m[h]	3.3—3.4m	4.4—4.8m	4.6—4.75m	4.5—4.6m	4.6m
$^{\beta}$C*H*$_2$$^{\gamma}$C*H*$_2$CO	1.6—2.5m[i]	1.8—2.8m[i]	1.9—2.7m[i]	1.9—2.6m[i]	1.8—2.6m[i]	2.0—2.5m[i]
OCMe$_3$	1.45	1.4—1.5	1.4—1.5	1.4—1.5	1.4—1.5	
C$_6$H$_4$(2′-H)			7.9—8.0d (8.5)	7.6—7.8d (8.5)	7.7—7.8d (9)	7.75d (9)
C$_6$H$_4$(3′-H)			7.3—7.35d (8.5)	6.4—6.6d (8.5)	6.7—6.8d (9)	6.75d (9)
NCH$_3$			3.35—3.4	2.85[j]	3.15—3.25	3.2
N*H*CH$_3$				*ca.* 4m		
Pteridine (7-H)					8.5—8.6	8.6

[a] Measured at 90 or 100 MHz, with chemical shifts δ downfield from SiMe$_4$. Signals are singlets unless otherwise stated; d refers to doublet, t to triplet, and m to multiplet *or* broad signal. Signals for amide *N*-alkyl groups are as follows: For compounds of the b series (CH_3), δ 2.75d (5 Hz); c, series (CH_2CH_3), 3.25d (5.5) of quartet (7), 1.1 t (7); d series ($CH_2CH_2CH_3$), 3.2m, 1.5m, 0.9t (7); e series ($CHMe_2$), 4.05m, 1.15d (6.5), 1.1d (6.5); f series ($CH_2CH_2CH_2CH_3$), 3.25m, 1.2—1.6m, 0.9m; g series (CH_2CHMe_2), 3.05m, 1.8m, 0.85d (6.5); h series ($CHMeCH_2CH_3$), 3.85m, 1.45m, 1.1d (6.5), 1.05d (6.5), 0.9m; i series (CMe_3), 1.35; j series (CH_2Ph), 4.4d (6),[j] 7.25; k series (C_6H_{11}), 3.65m, 1.7m, 1.25; l series (–$CH_2CH_2CH_2CH_2CH_2$–), 3.45m, 1.3—1.8m; m series (–$CH_2CH_2OCH_2CH_2$–) 3.2—3.8m. [b] In $CDCl_3$. [c] In CD_3OD–$CDCl_3$ (1:10). [d] In CD_3OD–$CDCl_3$ (1:2) (NH not observed). [e] In CD_3OD (NH not observed). [f] Signal absent in the *NN*-disubstituted amides. [g] For unsubstituted and monosubstituted α-monoamides (**11a—k**) and γ-monoamides (**21a—m**). [h] For *NN*-disubstituted α-monoamides (**11l**) and (**11m**) only. [i] For the α-monoamides (**11**)—(**15**), the signals of the β and γ protons appeared at 90—100 MHz as two distinct complexes, the latter resonances being more downfield. Of the γ-monoamides (**21**)—(**25**), only the *NN*-disubstituted amides showed separated resonances for the β and γ protons. [j] For the α-benzylamide (**14j**), decoupling showed that NHC*H*$_2$Ph was the AB part of an ABX spectrum with J_{gem} 13 and J_{NHCH} 6 Hz; NCH_3 resonated at δ 2.8.

ml). After completion of addition, the mixture was stirred for a further 1 h, and enough water was added to produce a clear solution. The residue obtained on evaporation of the solution to near dryness was dissolved in ethyl acetate (150 ml), and the solution was washed with water (2 × 50 ml) and saturated aqueous sodium chloride (100 ml), and dried over sodium sulphate. The oil obtained on evaporation of ethyl acetate was chromatographed over silica to give N-[p-*methyl(trifluoroacetyl)aminobenzoyl*]-L-*glutam-α-ethylamide* {N′-*ethyl*-N-[p-*methyl(trifluoroacetyl)aminobenzoyl*]-L-*isoglutamine*} *t-butyl ester* (**13c**) (4.2 g, 82%) as a gum (Found: *M*H$^+$ by H_2 c.i.m.s., 460.205 $C_{21}H_{29}F_3N_3O_5$ requires *M*H, 460.206).

Starting with the appropriate monoamide of t-butyl L-glutamate, the following monoamides of t-butyl *N*-[*p*-methyl-(trifluoroacetyl)aminobenzoyl]-L-glutamate were prepared by the same method to yield gums which gave, in the H_2 c.i.m.s., *M*H$^+$ and/or (*M*H – C_4H_8)$^+$ ions within 0.002 a.m.u. of the required masses: α-*methylamide* (**13b**) ($C_{20}H_{27}F_3N_3O_5$ and $C_{16}H_{19}F_3N_3O_5$ require *m/z* 446.190 and 390.128); α-*isopropylamide* (**13e**) ($C_{22}H_{31}F_3N_3O_5$ and $C_{18}H_{23}F_3N_3O_5$ require *m/z* 474.221 and 418.519); α-*butylamide* (**13f**); α-*isobutylamide* (**13g**); α-*sec-butylamide* (**13h**); α-*t-butylamide* (**13i**) (for the above four, $C_{23}H_{33}F_3N_3O_5$ and $C_{19}H_{25}F_3N_3O_5$ require *m/z* 488.237 and 432.174); and γ-*morpholide* (**23m**) ($C_{23}H_{31}F_3N_3O_6$ and $C_{19}H_{23}F_3N_3O_6$ require *m/z* 502.217 and 446.154). The α-*sec-butylamide* (**13h**) showed *m/z* (e.i.m.s.) 414 (3%, *M* – OC_4H_9), 388.161 (11, *M*H$^+$ – $CONHC_4H_9$), 332 (21), 331.093 (30, *b*), 231 (12), and 230.045 (100, $CF_3CONHMeC_6H_4CO^+$). Also prepared were the γ-*piperidide* derivative (**23l**) (*M*$^+$ by e.i.m.s., 499.229. $C_{24}H_{32}F_3N_3O_5$ requires *M*, 499.229), and the γ-*propylamide* derivatives (**23d**) which was obtained crystalline (from ether–light petroleum), m.p. 119—120 °C (Found: C, 56.2; H, 6.55; N, 8.5. $C_{22}H_{30}F_3N_3O_5$ requires C, 55.8; H, 6.4; N, 8.85%).

Monoamides of t-Butyl N-(p-*Methylaminobenzoyl*)-L-*glutamate.*—A solution of *N*-[*p*-methyl(trifluoroacetyl)aminobenzoyl]-L-isoglutamine t-butyl ester (**13a**) (6.35 mmol) in a 2:1:1 mixture of triethylamine, methanol and water (40 ml) was refluxed for 1 h. On reduction of volume under reduced pressure to 10 ml a precipitate was obtained which was filtered off to give N-(p-*methylaminobenzoyl*)-L-*isoglutamine t-butyl ester* (**14a**) as a low melting solid (1.36 g, 65%), *m/z* (e.i.m.s.) 335.184 (3%, *M*$^+$) ($C_{17}H_{25}N_3O_4$ requires *M*, 335.184), 235.108 (11, *b*), and 134.060 (100, $MeNHC_6H_4CO^+$).

In a similar manner and from the corresponding trifluoroacetyl derivatives, the following monoamides of *N*-(*p*-methylaminobenzoyl)-L-glutamic acid t-butyl ester were obtained after purification by chromatography over silica: γ-*amide* (**24a**), *m/z* (e.i.m.s.) 335.185 (3%, *M*$^+$) ($C_{17}H_{25}N_3O_4$ requires *M*, 335.184), 279.122 (4, *M* – C_4H_8), 262.119 (3, *M* – C_4H_9O), 201.124 (8, *M* – $MeNHC_6H_4CO$), and 134.060 (100, $MeNHC_6H_4CO^+$); α-*propylamide* (**14d**), m.p. 61—63 °C (Found: C, 63.15; H, 8.45; N, 10.95. $C_{20}H_{31}N_3O_4$ requires C, 63.65; H, 8.3; N, 11.15%); α-*butylamide* (**14f**), m.p. 67—69 °C (from ether–light petroleum) (Found: C, 62.6; H, 8.35; N, 10.55. $C_{21}H_{33}N_3O_4{\cdot}0.5H_2O$ requires C, 63.0; H, 8.55; N, 10.5%); α-*benzylamide* (**14j**), m.p. 128—129 °C (Found: C, 67.8; H, 7.3; N, 9.8. $C_{24}H_{31}N_3O_4$ requires C, 67.75; H, 7.35; N, 9.9%); α-*cyclohexylamide* (**14k**), m.p. 143—145 °C (from ethanol) (Found: C, 66.3; H, 8.55; N, 10.1%. $C_{23}H_{35}N_3O_4$ requires C, 66.15; H, 8.45; N, 10.05%); and γ-*cyclohexylamide* (**24k**), m.p. 168—169 °C (from ethyl acetate) (Found: C, 66.1; H, 8.45; N, 9.95%). Also prepared were the following monoamides of *N*-(*p*-methylaminobenzoyl)-L-glutamic acid t-butyl ester, obtained as purified gums, which were used directly in the coupling reaction described below: α-methylamide (**14b**), α-ethylamide (**14c**), γ-propylamide (**24d**), α-isopropylamide (**14e**), α-isobutylamide (**14g**), α-sec-butylamide (**14h**), α-t-butylamide (**14i**), γ-piperidide (**24l**), and γ-morpholide (**24m**).

Monoamides of t-Butyl N-[p-(2,4-*Diaminopteridin*-6-*yl*)-*methyl*(*methyl*)*aminobenzoyl*]-L-*glutamate.*—(*a*) To a stirred

Table 9. U.v. spectral data of methotrexate analogues[a]

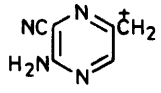
Compound	$\lambda_{max.}$	$\varepsilon \times 10^3$	$\lambda_{max.}$	$\varepsilon \times 10^3$	$\lambda_{max.}$	$\varepsilon \times 10^3$
(15a)	262	21.9	301	23.6	374	6.4
(15b)	263	22.3	303	24.2	377	7.4
(15c)	265	30.1	305	33.3	378	9.9
(15d)	262	26.7	303	29.7	376	8.7
(15e)	264	25.3	304	28.2	378	8.5
(15f)	265	24.1	305	25.5	377	8.1
(15g)	267	24.6	306	27.0	378	8.2
(15h)	263	25.6	303	28.4	377	8.5
(15i)	263	20.3	304	22.5	377	6.7
(15j)[b]	264	29	303	32	378	9.8
(25a)	261	22.7	301	24.3	374	5.9
(25l)	264	27.0	304	30.2	378	9.0
(25m)	264	21.9	301	23.3	378	7.2
(16a)	261	14.8	307	18.6	380	5.1
(16b)	260	25.0	307	26.5	372	8.7
(16c)	262	28.0	307	29.5	372	9.7
(16d)	260	22.9	305	21.8	374	8.2
(16e)	260	26.3	307	27.1	374	9.2
(16f)	261	26.9	308	28.1	374	9.2
(16g)	261	22.3	307	23.5	374	7.8
(16h)	261	21.2	307	22.2	374	7.4
(16i)	261	22.9	307	23.8	374	7.8
(16j)	262	19.1	308	19.8	374	6.5
(26a)	259	17.1	304	17.9	370	5.3
(26l)	261	28.6	306	28.0	373	9.6
(26m)	262	22.6	304	21.5	374	7.8

[a] $\lambda_{max.}$ in nm, solvent being methanol for the t-butyl esters (**15**) and (**25**), and aqueous sodium hydrogen carbonate for the acids (**16**) and (**26**) [saturated for acids (**16a**) and (**26a**), 0.01M for the other acids]. In the calculation of ε, the varying degree of hydration (see elemental analyses) has not been taken into account. [b] Molecular weight of $CHCl_3$–solvate used in calculation of ε.

solution of *N*-(*p*-methylaminobenzoyl)-L-glutam-α-ethylamide [*N'*-ethyl-*N*-(*p*-methylaminobenzoyl)-L-isoglutamine] t-butyl ester (**14c**) (2.05 g, 5.65 mmol) in dry *NN*-dimethylformamide (DMF) (25 ml) heated to 65—70 °C was added 2,4-diamino-6-bromomethylpteridine hydrobromide (**17**)[16] (2.47 g, 7.34 mmol). After 2.5 h, the reaction mixture was filtered and the solvent removed at 0.5 Torr and 60—70 °C. The viscous residue was taken up in methanol (50 ml) containing triethylamine (5 ml), and the volume was reduced to 5 ml. A mixture (50 ml) of methanol and chloroform (1:1) was added, and the solution was chromatographed over alumina to yield N-[p-(2,4-*diaminopteridin*-6-*yl*)*methyl*(*methyl*)*aminobenzoyl*]-L-*glutam*-α-*ethylamide* {N-[p-(2,4-*diaminopteridin*-6-*yl*)methyl-(methyl)aminobenzoyl]-N'-*ethyl*-L-*isoglutamine*} *t-butyl ester* (**15c**) as yellow needles (1.54 g, 51%) from ethanol–water, m.p. 135—137 °C (Found: C, 55.5; H, 6.4; N, 22.75. $C_{26}H_{35}N_9O_4 \cdot H_2O$ requires: C, 56.2; H, 6.7; N, 22.7%).

Starting with the appropriate monoamide of t-butyl *N*-(*p*-methylaminobenzoyl-L-glutamate and using the same method, the following monoamides of t-butyl *N*-[*p*-(2,4-diaminopteridin-6-yl)methyl(methyl)aminobenzoyl]-L-glutamate were prepared as yellow crystals: α-*propylamide* (**15d**), m.p. 216—218 °C (from ethanol–water) (Found: C, 54.5; H, 6.3; N, 21.1. $C_{27}H_{37}N_9O_4 \cdot 2.5H_2O$ requires C, 54.35; H, 7.1; N, 21.1%); γ-*propylamide* (**25d**) m.p. 160—162 °C (from ethanol–ethyl acetate) (Found: C, 58.5; H, 6.8; N, 22.5. $C_{27}H_{37}N_9O_4$ requires C, 58.8; H, 6.75; N, 22.85%); α-isopropylamide (**15e**), m.p. 138—139 °C (from ethanol–water) Found: C, 58.4; H, 6.25; N, 22.45%); α-*butylamide* (**15f**), m.p. 155—158 °C (from ethanol–water) (Found: C, 54.35; H, 6.6; N, 21.1. $C_{28}H_{39}N_9O_4 \cdot 2.5H_2O$ requires C, 55.05; H, 7.25; N, 20.95%); α-*isobutylamide* (**15g**), m.p. 123—126 °C (from ethanol–water) (Found: C, 59.4; H, 7.15; N, 21.1. $C_{28}H_{39}N_9O_4$ requires C, 59.45; H, 6.95; N, 22.3%); α-*sec-butylamide* (**15h**), m.p. 129—131 °C (from ethanol–water) (Found: C, 58.7; H, 6.9; N, 22.0. $C_{28}H_{39}N_9O_4 \cdot 0.5H_2O$ requires C, 58.5; H, 7.0; N, 21.95%); α-*t-butylamide* (**15i**), m.p. 146—147 °C (from ethanol–water) (Found: C, 58.35; H, 6.7; N, 21.7%. $C_{28}H_{39}N_9O_4 \cdot 0.5H_2O$ requires C, 58.5; H, 7.0; N, 21.95%); α-*benzylamide* (**15j**) m.p. 203—205 °C (from methanol–chloroform) (Found: C, 53.9; H, 5.55; N, 16.7. $C_{31}H_{37}N_9O_4 \cdot CHCl_3$ requires C, 53.45; H, 5.3; N, 17.5%); α-*cyclohexylamide* (**15k**), m.p. 126—129 °C (from ethanol–water) (Found: C, 60.7; H, 7.15. $C_{30}H_{41}N_9O_4$ requires C, 60.9; H, 7.0%); and γ-*piperidide* (**25l**), m.p. 173—175 °C (from methanol–chloroform) (Found: C, 60.2; H, 6.85; N, 22.75. $C_{29}H_{39}N_9O_4$ requires C, 60.3; H, 6.8; N, 22.8%). The following, obtained as yellow foams, were characterized after removal of the t-butyl ester group (see below): α-methylamide (**15b**), γ-cyclohexylamide (**25k**), and γ-morpholide (**25m**). The u.v. absorption data of the above products are given in Table 9.

(*b*) *N*-(*p*-Methylaminobenzoyl)-L-glutamine t-butyl ester (**24a**) (1.63 g, 4.85 mmol) was dissolved in ethanol (100 ml) containing triethylamine (0.77 ml, 5.34 mmol). A solution of 2-amino-5-bromomethyl-3-cyanopyrazine 1-oxide (**27**)[17] (1.22 g, 5.34 mmol) in ethanol (50 ml) was added dropwise to the stirred solution during 1 h. The solvent was evaporated and the residue partitioned between ethyl acetate (100 ml) and water (200 ml). The aqueous phase was extracted with ethyl acetate (3 × 100 ml), and the combined organic layers were washed successively with saturated aqueous sodium hydrogen carbonate and aqueous sodium chloride (100 ml each) and dried (sodium sulphate). The solvent was evaporated and the resulting residue was chromatographed on silica gel to give *N*-[*p*-(5-amino-6-cyano-4-oxidopyrazin-2-yl)methyl(methyl)amino-benzoyl]-L-glutamine t-butyl ester (**28a**) as a yellow gum (1.72 g, 73%), $\lambda_{max.}$(MeOH) 253 (ε 10 900), 299 (14 300), and 376 nm (3 100). The *N*-oxide (**28a**) (1.60 g) was deoxygenated by being heated with freshly distilled triethyl phosphite (10 ml) in DMF (90 ml) at 145—150 °C for 2.5 h. The residue obtained upon removal of solvents under reduced pressure was chromatographed over silica gel to give *N*-[*p*-(5-amino-6-cyanopyrazin-2-yl)methyl(methyl)aminobenzoyl]-L-glutamine t-butyl ester (**29a**) (0.87 g, 56%) as a yellow gum, $\lambda_{max.}$(MeOH) 251 (ε 12 300), 302 (23 900), and 357 nm (5 000); *m/z* (CH_4 c.i.) 468 (2%, MH^+), 412 (1, $MH - C_4H_8$), 266 (1, *M* − butyl glutamine), 151 (100, $MeNH_2{}^+C_6H_4CONH_2$), and 133 [60, ion (**33**)]. To a solution

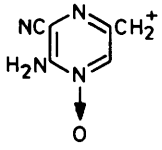


(**33**)

of this product (0.74 g, 1.56 mmol) in anhydrous t-butyl alcohol (150 ml) under reflux was added a solution of guanidine in t-butyl alcohol [prepared by adding potassium t-butoxide in t-butyl alcohol (3.1 ml, 0.51M) to guanidine hydrochloride (0.326 g, 3.50 mmol) and removing potassium chloride by centrifugation]. After a further 45 min of reflux, the hot solution was poured into water (600 ml), and after being saturated with sodium chloride was extracted with ethyl acetate (4 × 100 ml). The combined extracts were washed successively with saturated aqueous sodium hydrogen carbonate (200 ml) and saturated aqueous sodium chloride (200 ml) and was dried (sodium sulphate). The residue obtained on evaporation was chromatographed on silica gel to yield N-[p-(2,4-*diaminopteridin*-6-*yl*)*methyl*(*methyl*)*aminobenzoyl*]-L-*glutamine t-butyl ester* (**25a**) as needles from chloroform–methanol (53% yield),

m.p. 140—143 °C; for $\lambda_{max.}$ see Table 9 (Found: C, 54.3; H, 6.05; N, 23.35. $C_{24}H_{31}N_9O_4 \cdot H_2O$ requires C, 54.65; H, 6.3; N, 23.9%).

By the same three-step process, and starting with respectively the t-butyl esters of *N*-(*p*-methylaminobenzoyl)-L-isoglutamine (**14a**) and of *N*-(*p*-methylaminobenzoyl)-L-glutam-α-propylamide [*N*-(*p*-methylaminobenzoyl)-*N'*-propyl-L-isoglutamine] (**14d**), one obtained N-[p-(2,4-*diaminopteridin-6-yl*)*methyl*-(*methyl*)*aminobenzoyl*]-L-*isoglutamine t-butyl ester* (**15a**) and *N*-[*p*-(2,4-diaminopteridin-6-yl)methyl(methyl)aminobenzoyl]-L-glutam-α-propylamide {*N*-[*p*-(2,4-diaminopteridin-6-yl)methyl(methyl)aminobenzoyl]-*N'*-propyl-L-isoglutamine} t-butyl ester (**15d**). The former product (**15d**) was obtained as yellow crystals from methanol. m.p. 156—158 °C; for $\lambda_{max.}$ see Table 9 (Found: C, 53.2; H, 6.1; N, 22.9. $C_{24}H_{31}N_9O_4 \cdot 2H_2O$ requires C, 52.85; H, 6.45; N, 23.1%). The latter product (**15d**) was identical with the sample prepared by method (*a*) above. Pyrazine oxide intermediate (**18d**) gave *m/z* (CH_4 c.i.) 526 (4%, MH^+), 510 (10), 378 (20, M − 148), 322 (20, 378 − C_4H_8), 263 (20, 322 − $C_3H_7NH_2$), and 149 [100, ion (**34**)]. For ^{13}C n.m.r.



(**34**)

data of pyrazine intermediates (**28a**), (**29a**), (**19a**), and (**18d**), see Table 6.

α-and α-Monoamides of Methotrexate.—α-sec-Butylamide t-butyl ester (**15h**) (0.61 g, 1.07 mmol) was dissolved in anhydrous trifluoroacetic acid (15 ml) and the solution was stirred for 15 min. The solvent was evaporated, and the residue dissolved in 2M aqueous ammonium hydroxide (25 ml). The solution was filtered and the filtrate acidified to pH 4 with 2M hydrochloric acid. The precipitate was centrifuged, aggregated by being heated and cooled, and filtered to yield N-[p-(2,4-*diaminopteridin-6-yl*)*methyl*(*methyl*)*aminobenzoyl*]-N'-*sec-butyl-L-isoglutamine* (methotrexate-α-sec-butylamide) (**16h**) (0.48 g, 87%) as an orange solid, m.p. 180—182 °C (Found: C, 53.25; H, 6.05; N, 23.1. $C_{24}H_{31}N_4O_2 \cdot 2H_2O$ requires C, 52.85; H, 6.45; N, 23.1%).

From the corresponding t-butyl esters and by the same method, the following monoamides of methotrexate were prepared: α-amide (**16a**),[7] m.p. >300 °C; γ-amide (**26a**),[7] m.p. 195—198 °C (Piper *et al.*[7] did not record m.p.); *α-methylamide* (**16b**), m.p. 265—270 °C (Found: C, 51.5; H, 5.3; N, 25.8. $C_{21}H_{25}N_9O_4 \cdot H_2O$ requires C, 51.95; H, 5.6; N, 25.95%); *α-ethylamide* (**16c**), m.p. 275—276 °C (Found: C, 54.6; H, 5.7; N, 25.85. $C_{22}H_{27}N_9O_4$ requires C, 54.9; H, 5.65; N, 26.2%), *α-propylamide* (**16d**), m.p. 169—172 °C (Found: C, 51.8; H, 6.3; N, 24.0. $C_{23}H_{29}N_9O_4 \cdot 2H_2O$ requires C, 51.95; H, 6.25; N, 23.7%); *γ-propylamide* (**26d**), m.p. 175—179 °C (recrystallized from water) (Found: C, 51.65; H, 6.1; N, 23.6. $C_{23}H_{29}N_9O_4 \cdot 2H_2O$ requires C, 51.95; H, 6.25; N, 23.7%); *α-isopropylamide* (**16e**), m.p. 178—180 °C (Found: C, 52.0; H, 5.85; N, 23.6. $C_{23}H_{29}N_9O_4 \cdot 2H_2O$ requires C, 51.95; H, 6.25; N, 23.7%); *α-butylamide* (**16f**), m.p. 164—166 °C (Found: C, 53.4; H, 6.4; N, 23.1. $C_{24}H_{31}N_9O_4 \cdot 1.5H_2O$ requires C, 53.7; H, 6.4; N, 23.5%); *α-isobutylamide* (**16g**), m.p. 168—170 °C (Found: C, 53.3; H, 6.05; N, 23.25. $C_{24}H_{31}N_9O_4 \cdot 1.5H_2O$ requires C, 53.7; H, 6.4; N, 23.5); *α-t-butylamide* (**16i**), m.p. 179—182 °C (Found: C, 53.35; H, 5.9; N, 23.05. $C_{23}H_{31}N_9O_4 \cdot 1.5H_2O$ requires C, 53.7; H, 6.4; N, 23.5%); *α-benzylamide* (**16j**), m.p. 176—178 °C (Found: C, 57.35; H, 5.55; N, 21.9. $C_{27}H_{29}N_9O_4 \cdot H_2O$ requires C, 57.75; H, 5.55; N, 22.45%); *α-cyclohexylamide* (**16k**) as the hydrochloride salt, m.p. 220 °C (decomp.) (Found: C, 53.95; H, 5.8; N, 21.0. $C_{26}H_{33}N_9O_4 \cdot HCl$ requires C, 54.55; H, 5.95; N, 22.0%); *γ-cyclohexylamide* (**26k**) as the hydrochloride salt, m.p. 116—118 °C (Found: C, 52.9; H, 6.5; N, 20.45. $C_{26}H_{33}N_9O_4 \cdot HCl \cdot H_2O$ requires C, 52.9; H, 6.1; N, 21.35%); *γ-piperidide* (**26l**), m.p. 195—197 °C (Found: C, 55.6; H, 6.35; N, 22.75. $C_{25}H_{31}N_9O_4 \cdot H_2O$ requires C, 55.65; H, 6.15; N, 23.35%); and *γ-morpholide* (**26m**), m.p. 213—215 °C (Found: C, 52.7; H, 5.25; N, 22.1. $C_{24}H_{29}N_9O_5 \cdot 1.5H_2O$ requires C, 52.2; H, 5.9; N, 23.0%).

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