Controlled Regioselective Anilide Formation from Aspartic and Glutamic Acid Anhydrides†

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The regioselectivity of the reaction of aniline with a series of *N*-protected aspartic and glutamic acid anhydrides was controlled through the choice of reaction solvent. In benzene, the formation of aspartic or glutamic acid α-anilides was favored, with α:(*β* or *γ*) regioselectivities as high as 100:0. On the other hand, in DMSO, the formation of either aspartic acid *β*-anilide or glutamic acid *γ*-anilide was favored, with α:(*β* or *γ*) regioselectivities as high as 0:100. This regioselectivity was not observed for alkylamines, amino acids, thiols, or alcohols. However, the high yields of the aniline reactions and the ability to specify their regioselectivities demonstrate the usefulness of our method for the formation of aspartic and glutamic acid anilide bonds.

Introduction

N-Protected glutamic and aspartic acid anhydrides are very useful intermediates in peptide synthesis. Formation of an intramolecular anhydride from the parent *N*-protected amino acid is easily achieved and activates carboxylate carbonyls toward nucleophilic attack. Following nucleophilic addition, one carboxylate is released from the parent anhydride in a form that is suitable for a subsequent coupling reaction. In this way, the use of anhydrides serves as a more direct alternative to the protection, activation, and deprotection of carboxylates required to make aspartate and glutamate derivatives. Nucleophilic attack that is not completely regiospecific for one carbonyl results however in a mixture of α and *β*/*γ* product isomers that must be separated and purified, reducing the yield and ease of synthesis of the desired derivative. For example, the reactions of Cbz-glutamic acid anhydride with glycine[1] and with lysine[2] have been reported to produce mixture of α and *γ* coupled dipeptide products, whereas Cbz-aspartic acid anhydride has been reported[3] to react with phenylalanine with good regioselectivity. Both glutamic and aspartic acid anhydrides, *N*-protected with Fmoc, Cbz, or Boc groups, have also been reported to react with methanol with moderate regioselectivity.[4,5] The reactions of Cbz-[6,7] and Boc-[8]aspartic acid anhydrides in benzyl alcohol have been reported to give the α-benzyl ester with good regioselectivity.

Recently we were interested in the preparation of a series of novel *p*-substituted *γ*-anilides of Cbz-glutamic acid. During our initial attempts to make the *p*-nitroanilide, we found that activation of the *γ*-carboxylic group of Cbz-glutamic acid α-esters (through formation of the acid chloride or reaction with DCC) and subsequent attack of *p*-nitroaniline, a poor nucleophile, resulted in the formation of Cbz-pyroglutamic acid esters rather than the desired anilides. (The activation of Fmoc-glutamic acid as the chloride has also been shown[9] to give the pyroglutamate.) Since the reaction of glutamic acid anhydride (protected with an *N*-phthalyl group) with *p*-nitroaniline has been shown[10] to give the desired *γ*-isomer with good regioselectivity, we elected to try to make our target compounds in a similar manner, through the direct reaction of Cbz-glutamic acid anhydride with a series of anilines. We discovered that the regioselectivity of this reaction is remarkably dependent upon the reaction solvent and allows preparation of either the α- or the *γ*-isomer in nearly quantitative yield with good to excellent regioselectivity. Our investigation of this selective reaction as well as its extension to aspartic acid anhydride and to other *N*-protecting groups (Fmoc and Boc) are reported herein.

Results and Discussion

For each reaction between an *N*-protected aspartic or glutamic acid anhydride with an aniline (Scheme 1), reaction progress was followed by TLC, and upon disappearance of starting material (usually within 20 min) the reaction mixture was characterized by ^{1}H NMR in order to determine the product regioisomer ratios. Identification of the α-, *β*-, and *γ*-isomers was based on the (∼0.5 ppm) difference between the chemical shifts of their anilido N−H protons. The products were then isolated and purified through flash column chromatography and characterized by ^{1}H and ^{13}C NMR and through elemental analysis. Finally, authentic α-, *β*-, and *γ*-anilides were prepared by independent, unambiguous synthesis[11] and used to confirm the validity of our ^{1}H NMR assignments. As can been seen from Tables 1 and 2, the regioselec-

† Abbreviations used: Boc = *tert*-butoxycarbonyl, Cbz = benzoxycarbonyl, Fmoc = 9-fluorenylmethoxycarbonyl, Asp = L-aspartic acid, Glu = L-glutamic acid (the aspartic and glutamic acids used throughout this study were always the L stereoisomers).

⊗ Abstract published in *Advance ACS Abstracts,* November 15, 1997.

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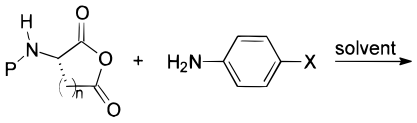
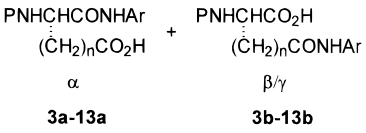
(10) Lindsay, H.; Whitaker, J. F. *Org. Prep. Proc. Int.* **1974**, *7*, 89−91 and unpublished observations from this laboratory.
(11) Independent synthetic route (not shown here) involved selective protection and deprotection of each carboxylate according to standard literature techniques.

S0022-3263(97)01375-3 CCC: $14.00

Scheme 1



1a (n = 2, P = Cbz) **2a** (X = H)
b (n = 2, P = Fmoc) **b** (X = CH_3)
c (n = 2, P = Boc) **c** (X = Cl)
d (n = 1, P = Cbz) **d** (X = OCH_3)
e (n = 1, P = Fmoc) **e** (X = NO_2)
f (n = 1, P = Boc)



3a-13a **3b-13b**

Table 1. Yields and Regioselectivities of the Reactions of *N*-Protected Glutamic and Aspartic Anhydrides with Various Anilines in Benzene

amino acid anhydride (*n*)	*N*-protecting group (P)	*p*-aniline substituent (X)	major product anilide	yield[a] (%)	regioselectivity (α:*β*/*γ*)[b]
Glu (2)	Cbz	H	**3a**	98	100:0
Glu (2)	Cbz	CH_3	**4a**	95	100:0
Glu (2)	Cbz	Cl	**5a**	94	100:0
Glu (2)	Cbz	OCH_3	**6a**	99	100:0
Glu (2)	Cbz	NO_2	**7a**	95	8:1
Glu (2)	Cbz	NO_2	**7a**	85	100:0[c]
Glu (2)	Fmoc	H	**8a**	84	100:0
Glu (2)	Boc	H	**9a**	95	11:1
Asp (1)	Cbz	H	**10a**	87	5:1
Asp (1)	Cbz	CH_3	**11a**	95	6:1
Asp (1)	Fmoc	H	**12a**	96	3:1
Asp (1)	Boc	H	**13a**	100	2:1

[a] Refers to combined masses of both isolated and purified anilide isomers. [b] As determined by ^{1}H NMR through integration of anilido N−H peaks. [c] In the presence of 2% acetic acid.

Table 2. Yields and Regioselectivities of the Reactions of *N*-Protected Glutamic and Aspartic Anhydrides with Various Anilines in DMSO

amino acid anhydride (*n*)	*N*-protecting group (P)	*p*-aniline substituent (X)	major product anilide	yield[a] (%)	regioselectivity (α:*β*/*γ*)[b]
Glu (2)	Cbz	H	**3b**	93	1:18
Glu (2)	Cbz	CH_3	**4b**	99	1:12
Glu (2)	Cbz	Cl	**5b**	99	1:11
Glu (2)	Cbz	OCH_3	**6b**	99	1:11
Glu (2)	Fmoc	H	**8b**	97	0:100
Glu (2)	Boc	H	**9b**	95	1:7
Asp (1)	Cbz	H	**10b**	93	1:4
Asp (1)	Cbz	CH_3	**11b**	92	1:4
Asp (1)	Fmoc	H	**12b**	95	1:6
Asp (1)	Boc	H	**13b**	100	1:2

[a] Refers to combined masses of both isolated and purified anilide isomers. [b] As determined by ^{1}H NMR through integration of anilido N−H peaks.

tivities thus determined for the reactions examined are excellent and attest to the usefulness and economy of our method for making either α- or *β*-/*γ*-anilides of aspartic or glutamic acids.

The reaction of *N*-protected glutamic (**1a**−**c**) or aspartic (**1d**−**f**) acid anhydrides with anilines **2a**−**e** in a nonpolar solvent such as benzene affords the α-anilide isomers (**3a**−**13a**) with high regioselectivity in excellent yields, as shown in Table 1. Notably, the reaction of Cbz-glutamic acid anhydride with anilines gave the α-isomer anilide exclusively.[12] When the protecting group was changed from Cbz to Fmoc, the ring opening in benzene was still found to give exclusively α-anilides. However, in the case of the Boc-glutamic acid anhydride, a small amount of *γ*-anilide was also formed, although the α-anilide remains the major product.[13] When the same reactions were performed with *N*-protected aspartic rather than glutamic acid anhydrides, the regioselectivities were good, but not excellent, as might be expected of a more reactive anhydride.[14]

In sharp contrast to the results in benzene, reactions in a polar aprotic solvent such as DMSO gave predominantly the *γ*- or *β*-isomers (**3b**−**13b**) in excellent yields. For example, the reaction of Cbz- or Fmoc-glutamic acid anhydride with anilines produced almost exclusively the *γ*-anilide isomer (Table 2). The more reactive aspartic acid anhydride analogues gave somewhat lower regioselectivity, with the *β*-isomer as the major product. The regioselectivities of the reactions of the aspartic and glutamic acid anhydrides protected with a Boc group were found once again to be lower than those of their Cbz- and Fmoc-protected analogues; the regioselectivity of the reaction of Boc-glutamic acid anhydride was moderate, and that of Boc-aspartic acid anhydride was poor.

The possibility of exploiting this method through the use of other nucleophiles was also explored. However, reaction of *N*-protected aspartic and glutamic acid anhydrides with thiols such as thiophenol and benzyl mercaptan (chosen for their resemblance to aniline) showed no regioselectivity. Phenol and cyclohexanol were unreactive, whereas alcohols such as benzyl alcohol, allyl alcohol, and methanol[15] reacted with poor regioselectivity. In reactions of the respective alkoxides with *N*-protected glutamic acid anhydride, the alkoxides appeared to react as bases rather than as nucleophiles, catalyzing the formation of pyroglutamic acid. In addition, reactions of primary alkylamines and α-amino acids proceed without regioselectivity. Earlier work[16] has suggested that there is no relationship between the steric and electronic nature of primary amines and the α/*γ* product distribution from their nucleophilic attack on Cbz-glutamic acid anhydride. The greater regioselectivity of anilines compared to primary alkylamines may be due to their reduced basicity (nucleophilicity) and greater steric bulk.

Other workers have also observed similar solvent effects on the regioselectivity of *N*-protected aspartic or glutamic acid anhydride ring opening. Yang reported[3] that the reaction of Cbz-aspartic acid anhydride with phenylalanine in DMSO gave predominantly the *β*-isomer dipeptide (*β*:α = 25:1). However, in toluene the regioselectivity of the same reaction was reversed, and the major product was the α-isomer (α:*β* = 3:1). Benoiton

(12) A noteworthy exception is the case of *p*-nitroaniline, where the regioselectivity of 8:1 (α:*γ*) was improved to exclusive α-isomer through the addition of a catalytic amount of acetic acid, probably due to activation of the α-carbonyl through selective protonation. It was also observed that many of the α-anilide products actually crystallized out of solution as the reaction progressed, further facilitating their regioselective synthesis through this modified procedure.

(13) The addition of a catalytic amount of acetic acid to the reaction of aniline with Boc-glutamic acid anhydride in boiling benzene did not enhance regioselectivity as it did in the case of *p*-nitroaniline attack on Cbz-glutamic acid anhydride (see ref 12), probably owing to the greater nucleophilicity of aniline compared to *p*-nitroaniline.

(14) Note however, that for the reaction of Cbz-aspartic acid anhydride with aniline, pure α-anilide isomer can be obtained from the reaction mixture by selective precipitation through the addition of Et_2O.

(15) See also refs 4 and 5.

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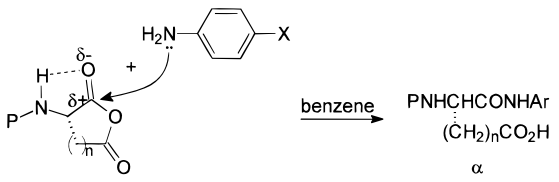
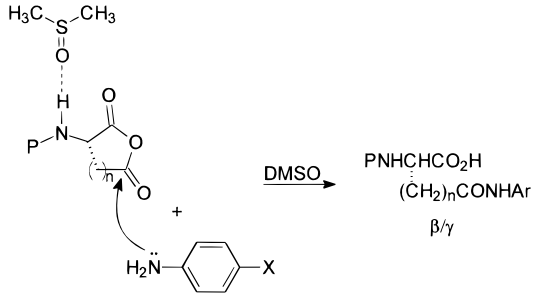
Scheme 2



Scheme 3



has also reported[4] a similar solvent effect and modest regioselectivity for the attack of phenylalanine on Cbz-aspartic acid anhydride. In DMF, the β-dipeptide was the predominant product (β:α = 5:1) whereas in dichloromethane, the α-isomer, was the major product (α:β = 2:1). All of these examples are consistent with our findings in that the use of a nonpolar reaction solvent favors formation of the α-isomer, whereas the use of a polar aprotic reaction solvent gives rise to the β- or γ-isomer.

One possible reason for this effect on regioselectivity may be the involvement of an intramolecular hydrogen bond proposed[3] to exist between the hydrogen on the α-amino nitrogen and the oxygen of the α-carbonyl (Scheme 2). In nonpolar solvents, such an intramolecular interaction would be strong and render the α-carbonyl more electrophilic and more susceptible to nucleophilic attack. Table 1 shows that in benzene the α-anilide is indeed the predominant isomer. In polar aprotic solvents such as DMSO, the putative intramolecular hydrogen bond would be replaced by an intermolecular hydrogen bond between the α-amino hydrogen and a solvent molecule (Scheme 3). In the absence of intramolecular activation, attack is favored on the less sterically hindered β/γ, as illustrated in Table 2. For example, *N*-(phthalyl)glutamic acid anhydride, which lacks any hydrogen on the α-amino nitrogen and is thus incapable of forming an intramolecular hydrogen bond, reacts with anilines to give exclusively the γ-anilide isomers.[10]

The role of the steric environments of the α and β/γ carbonyls in the regioselectivity of the reaction is revealed in the influence of the size of the *N*-protecting group on regioselectivity. For example, anhydrides substituted with large *N*-protecting groups more capable of sterically hindering the α-carbonyl, such as Cbz and Fmoc, reacted with greater regioselectivity than those protected with the smaller Boc group (Tables 1 and 2). Very low regioselectivity has also been reported for the reaction of aniline with *N*-(acetyl)glutamic acid anhydride[17] and for the reaction of *p*-cyanoaniline with *N*-(trifluoroacetyl) aspartic[18,19] and glutamic[19] acid anhydrides. This effect of steric hindrance is more pronounced in the case of glutamic acid anhydrides than aspartic acid anhydrides because of the greater distance between the α-amino group and the ω-carbonyl.

Conclusions

We have shown that the regioselectivity of the reaction of anilines with Cbz- and Fmoc-aspartic and glutamic acid anhydrides can be controlled through choice of reaction solvent to give regioselectively α- or ω-anilides. In benzene, α-anilides are obtained, whereas in DMSO, β/γ-anilides are produced. Our method provides a direct and high-yielding alternative to multistep processes involving the selective protection, activation, substitution, and deprotection of aspartic and glutamic carboxylic acids. This reaction is therefore very useful for the formation of aspartic and glutamic anilides and should find its place among the repertoire of peptide chemists.

Experimental Section

Materials. L-Aspartic acid, L-glutamic acid, and *N*,*N*′-dicyclohexylcarbodiimide (DCC) were purchased from Sigma-Aldrich; the acylating reagents Cbz-Cl, Fmoc-Cl, and di-*tert*-butyl dicarbonate ("Boc anhydride") were purchased from Aldrich Chemical Co. Anilines were either purchased from Aldrich or prepared in our laboratory via a modified Hofmann rearrangement.[20,21] Benzene and DMSO were dried over CaH_2 prior to use. Other commercially available products were used without further purification. Column chromatography was carried out by using silica gel (200−430 mesh) obtained from A & C American Chemicals, Ltd. Analytical silica gel 60 F254 aluminum-backed TLC plates were purchased from EM Science and were developed using UV light (254 nm).

Methods. Reported melting points are uncorrected. Elemental analyses were performed on-site in the Laboratoire d'Analyse Élémentaire of the Université de Montréal.

L-*N*-Cbz-glutamic Acid α-Anilide (3a). General Procedure. Aniline (46 μL, 0.5 mmol) and L-*N*-Cbz-glutamic acid anhydride[2] (**1a**) (138 mg, 0.5 mmol) were heated in boiling benzene for 20 min. The pure product was collected by filtration as a white solid (175 mg, 98%) and washed with benzene. A sample was recrystallized for microanalysis from $CHCl_3$: mp 196−197 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00−2.14 (m, 1H), 2.18−2.22 (m, 1H), 2.42−2.57 (m, 1H), 2.62−2.74 (m, 1H), 4.58−4.70 (m, 1H), 5.15 (s, 2H), 5.70 (d, 1H, J = 6 Hz), 7.05−7.12 (m, 1H), 7.22−7.32 (m, 2H), 7.38 (s, 5H), 7.50−7.59 (m, 2H), 8.88 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.6, 172.8, 158.7, 139.5, 138.2, 129.9, 129.6, 129.2, 129.0, 125.6, 121.7, 68.0, 56.6, 31.4, 28.8. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.7; N, 7.9. Found: C, 64.4; H, 5.8; N, 7.8.

L-*N*-Cbz-glutamic acid α-*p*-methylanilide (4a): yield 95%; mp 178−180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.98−2.12 (m, 1H), 2.16−2.28 9m, 1H), 2.30 (s, 3H), 2.42−2.58 (m, 1H), 2.60−2.74 (m, 1H), 4.56−4.69 (m, 1H), 5.12 (s, 2H), 5.73 (d, 1H, J = 6 Hz), 7.00−7.12 (m, 2H), 7.30−7.48 (m, 7H), 8.72 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 172.5, 158.5, 138.1, 136.8, 135.3, 130.3, 129.5, 129.0, 128.9, 121.7, 67.8, 56.5, 31.3, 28.7, 20.9. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.9; H, 6.0; N, 7.6. Found: C, 65.2; H, 6.2; N, 7.5.

L-*N*-Cbz-glutamic acid α-*p*-chloroanilide (5a): yield 94%; mp 183−184 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00−2.12 (m, 1H), 2.15−2.28 (m, 1H), 2.40−2.54 (m, 1H), 2.60−2.74 (m, 1H), 4.55−4.68 (m, 1H), 5.15 (s, 2H), 5.68 (d, 1H, J = 6 Hz), 7.28 (s, 5H), 7.32−7.40 (m, 2H), 7.42−7.53 (m, 2H), 8.99 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.4, 172.7, 158.7, 138.3, 138.1, 130.3, 129.8, 129.5, 129.1, 128.9, 122.8, 67.9, 56.5, 31.2, 28.6. Anal. Calcd for $C_{19}H_{19}ClN_2O_5$: C, 58.4; H, 4.9; N, 7.2. Found: C, 58.5; H, 5.0; N, 7.0.

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L-*N*-Cbz-glutamic acid α-*p*-methoxyanilide (6a): yield 99%; mp 184−185 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00−2.14 (m, 1H), 2.18−2.30 (m, 1H), 2.42−2.55 (m, 1H), 2.59−2.62 (m, 1H), 3.78 (s, 3H), 4.55−4.68 (m, 1H), 5.13 (s, 2H), 5.76 (d, 1H, J = 6 Hz), 6.77−6.86 (m, 2H), 7.34 (s, 5H), 7.40−7.48 (m, 2H), 8.77 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 172.4, 158.5, 158.2, 138.1, 132.3, 129.5, 129.0, 128.9, 123.4, 145.0, 67.8, 56.4, 55.9, 31.3, 28.7. Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.2; H, 5.7; N, 7.2. Found: C, 62.1; H, 5.8; N, 7.2.

L-*N*-Cbz-glutamic Acid α-*p*-Nitroanilide (7a). After 2 h of heating in the presence of acetic acid (0.2 mL), a pale yellow solid (0.171 g, 85%) was obtained: mp 162−164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00−2.14 (m, 1H), 2.20−2.34 (m, 1H), 2.48−2.60 (m, 1H), 2.64−2.80 (m, 1H), 4.66−4.70 (m, 1H), 5.15 (s, 2H), 5.56 (d, 1H, J = 6 Hz), 7.38 (s, 5H), 7.66−7.74 (m, 2H), 8.10−8.20 (m, 2H), 9.52 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.4, 173.2, 158.5, 145.7, 144.8, 138.0, 129.5, 129.0, 128.9, 125.7, 120.7, 67.9, 56.6, 31.2, 28.4. Anal. Calcd for $C_{19}H_{20}N_3O_7$: C, 56.7; H, 5.0; N, 10.4. Found: C, 57.0; H, 4.9; N, 10.3. In the absence of acetic acid, a mixture of α and γ isomers was obtained (95% yield, α:γ = 8:1).

L-*N*-Cbz-glutamic Acid γ-Anilide (3b). General Procedure. L-*N*-Cbz-glutamic acid anhydride[2] (**1a**) (66, mg, 0.25 mmol) and aniline (27 μL, 0.30 mmol) were stirred in DMSO (2.0 mL) for 20 min at rt. Sodium bicarbonate (saturated, 10 mL) and water (10 mL) were then added, and the aqueous solution was extracted twice with CH_2Cl_2. The aqueous solution was then acidified to pH 1 and the product extracted with EtOAc (3 × 30 mL). The combined EtOAc layers were washed with water and dried over $MgSO_4$, and the solvent was removed to give a white solid (83 mg, 93%, γ:α = 18:1). Separation by flash column chromatography (eluent EtOAc, then 5% MeOH in EtOAc) afforded the pure γ-anilide as a white solid. A sample was recrystallized for microanalysis from EtOH/water: mp 157−158 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.16 (m, 1H), 2.28−2.40 (m, 1H), 2.48−2.73 (m, 2H), 4.36−4.48 (m, 1H), 5.12 (s, 2H), 5.91 (d, 1H, J = 6 Hz), 7.08−7.20 (m, 1H), 7.26−7.40 (m, 7H), 7.48−7.58 (m, 2H), 7.98 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.5, 173.3, 158.8, 139.9, 138.2, 129.9, 129.6, 129.1, 129.0, 125.3, 121.5, 67.9, 55.1, 34.3, 28.7. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.7; N, 7.9. Found: C, 64.3; H, 6.1; N, 8.1.

L-*N*-Cbz-glutamic acid γ-*p*-methylanilide (4b): yield 99% (γ:α = 11.6:1); mp 135−137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.20 (m, 1H), 2.26−2.42 (m, 1H), 2.32 (s, 3H), 2.48−2.72 (m, 2H), 4.37−4.50 (m, 1H), 5.12 (s, 2H), 5.92 (d, 1H, J = 6 Hz), 7.05−7.20 (m, 2H), 7.26−7.49 (m, 7H), 7.94 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.5, 173.2, 158.8, 138.2, 137.3, 135.0, 130.3, 129.6, 129.1, 129.0, 121.5, 67.8, 55.1, 34.3, 28.7, 21.0. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.9; H, 6.0; N, 7.6. Found: C, 64.5; H, 6.2; N, 7.6.

L-*N*-Cbz-glutamic acid γ-*p*-chloroanilide (5b): yield 99% (γ:α = 11.2:1); mp 162−164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.16 (m, 1H), 2.28−2.40 (m, 1H), 2.48−2.74 (m, 2H), 4.37−4.48 (m, 1H), 5.24 (s, 1H), 5.85 (d, 1H, J = 6 Hz), 7.25−7.45 (m, 7H), 7.45−7.58 (m, 2H), 8.12 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.4, 173.2, 158.7, 138.7, 138.0, 129.9, 129.8, 129.5, 129.0, 128.9, 122.6, 67.8, 55.0, 34.2, 28.5. Anal. Calcd for $C_{19}H_{19}ClN_2O_5$: C, 58.4; H, 4.9; N, 7.2. Found: C, 58.4; H, 5.0; N, 7.1.

L-*N*-Cbz-glutamic acid γ-*p*-methoxyanilide (6b): yield 98% (γ:α = 11.1:1); mp 118−120 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02−2.20 (m, 1H), 2.20−2.42 (m, 1H), 2.50−2.72 (m, 2H), 3.80 (s, 3H), 4.38−4.48 (m, 1H), 5.12 (s, 2H), 5.92 (d, 1H, J = 6 Hz), 6.48−6.92 (m, 2H), 7.32 (s, 5H), 7.34−7.45 (m, 2H), 7.84 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 175.5, 173.1, 158.8, 158.1, 138.3, 132.9, 129.6, 129.1, 129.0, 123.3, 115.1, 67.8, 56.0, 55.2, 34.2, 28.8. Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.2; H, 5.7; N, 7.2. Found: C, 62.1; H, 5.7; N, 7.2.

L-*N*-Fmoc-glutamic Acid α-Anilide (8a). L-*N*-Fmoc-glutamic acid anhydride[4] (**1b**) (176 mg, 0.5 mmol) and aniline (55 μL, 0.6 mmol) were heated in boiling benzene (20 mL) for 20 min. Hexane (40 mL) was then added, and the precipitate was collected by filtration to give the pure product as a white solid (186 mg, 84%). A sample was recrystallized for microanalysis from EtOH/H_2O: mp 213-215 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80−2.05 (m, 2H), 2.24−2.45 (m, 2H), 4.12−4.35 (m, 4H), 7.00−7.12 (m, 1H), 7.20−7.39 (m, 4H), 7.39−7.46 (m, 2H), 7.55−7.65 (m, 2H), 7.65−7.79 (m, 3H), 7.84−7.92 (m, 2H), 10.03 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 170.6, 156.1, 143.9, 140.8, 138.9, 128.7, 127.7, 127.1, 125.4, 123.4, 120.1, 119.4, 65.8, 54.8, 46.7, 30.4, 27.2. Anal. Calcd for $C_{26}H_{24}N_2O_5$: C, 70.3; H, 5.4; N, 6.3. Found: C, 69.9; H, 5.5; N, 6.1.

L-*N*-Fmoc-glutamic Acid γ-Anilide (8b). L-*N*-Fmoc-glutamic acid anhydride[4] (**1b**) (88.8 mg, 0.25 mmol) and aniline (27.3 μL, 0.30 mmol) in DMSO (3 mL) were stirred for 20 min at rt. Ethyl acetate was then added, and the solution was washed with water, then dilute HCl, and then dried over $MgSO_4$. Evaporation of the solvent gave the pure γ product (103.2 mg, 97%). A sample was recrystallized for microanalysis from EtOH/H_2O: mp 218−219 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.80−1.95 (m, 1H), 2.05−2.20 (m, 1H), 2.39−2.55 (m, 2H), 3.96−4.06 (m, 1H), 4.14−4.35 (m, 3H), 6.98−7.05 (m, 1H), 7.24−7.38 (m, 4H), 7.38−7.46 (m, 2H), 7.50−7.62 (m, 2H), 7.65−7.79 (m, 3H), 7.84−7.92 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.7, 170.4, 156.2, 143.9, 140.8, 139.3, 128.7, 127.7, 127.1, 125.3, 123.0, 120.2, 119.1, 65.7, 53.5, 46.7, 32.8, 26.5. Anal. Calcd for $C_{26}H_{24}N_2O_5$: C, 70.3; H, 5.4; N, 6.3. Found: C, 70.2; H, 5.5; N, 6.3.

L-*N*-Boc-glutamic Acid α-Anilide (9a). L-*N*-Boc-glutamic acid anhydride[22] (**1c**) was allowed to react with aniline according to the same procedure used for L-*N*-Cbz-glutamic acid α-anilide (**3a**). A mixture of products (α:γ = 11:1) was obtained (180 mg, 95%). Separation by flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1, then EtOAc, and then 5% EtOH in EtOAc) yielded both the pure α-isomer (0.165 g, 86%) and the pure γ-isomer (15 mg, 8%). A sample of the major product was recrystallized for microanalysis from EtOAc/hexane: mp 73−75 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.98−2.12 (m, 1H), 2.18−2.30 (m, 1H), 2.42−2.58 (m, 1H), 2.58−2.70 (m, 1H), 4.56−4.68 (m, 1H), 5.72 (d, 1H, J = 6 Hz), 7.00−7.12 (m, 1H), 7.18−7.32 (m, 2H), 7.50−7.62 (m, 2H), 9.26 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 170.8, 155.7, 138.9, 128.7, 123.4, 119.4, 78.3, 54.5, 30.4, 28.2, 27.2. Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.6; H, 6.9; N, 8.7. Found: C, 59.8; H, 7.2; N, 8.5.

L-*N*-Boc-glutamic Acid γ-Anilide (9b). L-*N*-Boc-glutamic acid anhydride[22] (**1c**) was allowed to react with aniline according to the procedure used for L-*N*-Cbz-glutamic acid γ-anilide (**3b**). Yield: 95% (γ:α = 7.2:1). The products were separated through flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1, then EtOAc, and then 5% EtOH in EtOAc). A sample of the major product was recrystallized for microanalysis from EtOAc/hexane: mp 182−184 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.00−2.12 (m, 1H), 2.22−2.40 (m, 1H), 2.42−2.70 (m, 2H), 4.30−4.45(m, 1H), 5.60 (d, 1H, J = 6 Hz), 7.08−7.20 (m, 1H), 7.25−7.32 (m, 2H), 7.50−7.64 (m, 2H), 8.49 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.2, 168.2, 155.3, 139.1, 128.7, 123.2, 119.1, 78.2, 50.2, 38.2, 28.2; ^{13}C NMR (100 MHz, CD_3OD) δ 177.3, 175.0, 159.8, 141.5, 131.4, 126.8, 123.0, 82.3, 56.2, 36.0, 30.4. Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.6; H, 6.9; N, 8.7. Found: C, 59.3; H, 7.1; N, 8.5.

L-*N*-Cbz-Aspartic Acid α-Anilide (10a). General Procedure. (a) L-*N*-Cbz aspartic acid anhydride[23] (**1d**) (124.5 mg, 0.5 mmol) and aniline (55 mL, 0.6 mmol) in benzene (5.0 mL) and AcOH (0.1 mL) were heated to reflux for 10 min. The mixture was then cooled to rt and Et_2O (40 mL) was added. The precipitate (148 mg, 58%, a only) was collected by filtration. A sample of the product was recrystallized for microanalysis from $CHCl_3$: mp 177−179 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.84 (dd, 2H, J_1 = 17.5, J_2 = 6.4 Hz), 3.05−3.20 (m, 2H), 4.68−4.70 (m, 1H), 5.19 (s, 2H), 6.09 (d, 1H, J = 6 Hz), 7.10−7.20 (m, 1H), 7.28−7.42 (m, 7H), 7.42−7.52 (m, 2H), 8.43 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.9, 171.7, 158.4, 139.4, 138.0, 129.8, 129.5, 129.0, 128.9, 125.5, 121.7, 67.9, 53.7, 37.2. Anal. Calcd for $C_{18}H_{18}N_2O_5$: C, 63.2; H, 5.3; N, 8.2. Found: C, 63.2; H, 5.6; N, 8.2.

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(b) If the precipitate in benzene was collected without addition of Et_2O and washed with benzene (10 mL), a mixture (171 mg, 87%, α:β = 3:1) was obtained.

L-*N*-Cbz-aspartic acid α-*p*-methylanilide (11a): **(a)** yield 67% (α only) by Et_2O precipitation; mp 175−177 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32(s, 3H), 2.82 (dd, 2H, J_1 = 17.3, J_2 = 6.4 Hz), 3.04−3.18 (m, 2H), 4.65−4.78 (m, 1H), 5.16 (s, 2H), 6.08 (d, 1H, J = 6 Hz), 7.05−7.18 (m, 2H), 7.30−7.45 (m, 7H), 8.48 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 173.9, 171.5, 158.3, 138.0, 136.7, 135.3, 130.2, 129.5, 129.0, 128.9, 121.8, 67.9, 53.7, 37.3, 20.9. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.7; N, 7.9. Found: C, 64.1; H, 5.7; N, 7.8.

(b) Without addition of Et_2O, a mixture was obtained (95%, α:β = 3:1).

L-*N*-Cbz-aspartic Acid β-Anilide (10b). General Procedure. L-*N*-Cbz-aspartic acid anhydride[23] (**1d**) was allowed to react with aniline according to the procedure used for L-*N*-Cbz-glutamic acid γ-anilide (**3b**). A mixture of products was obtained (93%, β:α = 4:1), and the major product was purified by flash column chromatography (eluent EtOAc, then 5% MeOH in EtOAc). A sample of the product was recrystallized for microanalysis from $CHCl_3$: mp 170−172 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.98 (dd, 2H, J_1 = 16.1, J_2 = 7.7 Hz), 3.08−3.22 (m, 2H), 4.55−4.64 (m, 1H), 5.14 (s, 2H), 6.15 (d, 1H, J = 6 Hz), 7.32−7.42 (m, 7H), 7.45−7.52 (m, 2H), 7.66 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.7, 170.6, 158.4, 139.7, 138.1, 129.8, 129.4, 129.0, 129.7, 125.3, 121.3, 67.7, 52.2, 39.6. Anal. Calcd for $C_{18}H_{18}N_2O_5$: C, 63.2; H, 5.3; N, 8.2. Found: C, 63.2; H, 5.6; N, 8.2.

L-*N*-Cbz-aspartic acid β-*p*-methylanilide (11b): yield 92% (β:α = 4.3:1). mp 177−179 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.32 (s, 3H), 2.93 (dd, 2H, J_1 = 15.6, J_2 = 7.7 Hz), 3.08−3.22 (m, 2H), 4.54−4.64 (m, 1H), 5.14 (s, 2H), 6.17 (d, 1H, J = 6 Hz), 7.10−7.20 (m, 2H), 7.30−7.40 (m, 7H), 7.72 (s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.8, 170.5, 158.4, 138.1, 137.0, 135.1, 130.3, 129.5, 129.0, 128.7, 121.5, 67.7, 52.3, 39.5, 21.0. Anal. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.0; H, 5.7; N, 7.9. Found: C, 64.2, H, 6.0; N, 7.7.

L-*N*-Fmoc-aspartic Acid α-Anilide (12a). L-*N*-Fmoc-aspartic acid anhydride[4] (**1e**) was allowed to react with aniline according to the procedure used for L-*N*-Fmoc-glutamic acid α-anilide (**8a**). Yield: 96% (α:β = 3:1). Purification of the major product through flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1 with 0.5% AcOH) gave pure α-isomer as a white solid. A sample of the product was recrystallized for microanalysis from EtOH/H_2O: mp 191−193 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60 (dd, 1H, J_1 = 16, J_2 = 8 Hz), 2.74 (dd, 1H, J_1 = 16, J_2 = 5 Hz), 4.18−4.38 (m, 3H), 4.48−4.55 (m, 1H), 7.00−7.10 (m, 1H), 7.20−7.38 (m, 4H), 7.38−7.48 (m, 2H), 7.55−7.64 (m, 2H), 7.80−7.88 (m, 2H), 7.88−7.83 (m, 1H), 7.83−7.94 (m, 2H), 10.09 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.6, 169.7, 155.9, 143.8, 140.8, 139.0, 128.7, 127.7, 127.1, 125.4, 123.4, 120.2, 119.4, 65.8, 52.2, 46.7, 36.3. Anal. Calcd for $C_{25}H_{22}N_2O_5$: C, 69.8; H, 5.2; N, 6.5. Found: C, 69.6; H, 5.2; N, 6.4.

L-*N*-Fmoc-aspartic Acid β-Anilide (12b). L-*N*-Fmoc-aspartic acid anhydride[4] (**1e**) was allowed to react with aniline according to the procedure used for L-*N*-Fmoc-glutamic acid γ-anilide (**8b**). Yield: 95% (β:α = 5.5:1). Purification of the major product through flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1 with 0.5% AcOH) gave pure β-isomer as a white solid. A sample of the product was recrystallized for microanalysis from EtOH/H_2O: mp 174−176 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.70 (dd, 1H, J_1 = 16, J_2 = 8 Hz), 2.86 (dd, 1H, J_1 = 16 Hz, J_2 = 5 Hz), 4.20−4.35 (m, 3H), 4.40−4.53 (m, 1H), 7.00−7.10 (m, 1H), 7.20−7.36 (m, 4H), 7.36−7.46 (m, 2H), 7.52−7.65 (m, 2H), 7.65−7.76 (m, 3H), 7.85−7.92 (m, 2H), 10.00 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.1, 168.1, 155.9, 143.8, 140.7, 139.2, 128.7, 127.7, 127.1, 125.3, 123.2, 120.2, 119.1, 65.7, 50.5, 46.6, 38.2. Anal. Calcd for $C_{25}H_{22}N_2O_5$: C, 69.8; H, 5.2; N, 6.5. Found: C, 70.0; H, 5.5; N, 6.6.

L-*N*-Boc-aspartic Acid α-Anilide (13a). L-*N*-Boc-aspartic acid anhydride[24] (**1f**) was allowed to react with aniline according to the procedure used for L-*N*-Cbz-glutamic acid α-anilide (**3a**). Yield: 100% (α:β = 2:1). Isolation of the pure α-isomer by flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1, then EtOAc, and then 5% MeOH in EtOAc) gave a white solid. A sample of the product was recrystallized for microanalysis from EtOAc/hexane: mp 160−161 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.84 (dd, 1H, J_1 = 17 Hz, J_2 = 6 Hz), 3.00−3.16 (m, 1H), 4.60−4.78 (m, 1H), 5.75−5.90 (m, 1H), 7.07−7.16 (m, 1H), 7.20−7.38 (m, 2H), 7.46−7.55 (m, 2H), 8.62 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 177.7, 168.8, 159.8, 141.1, 131.5, 127.2, 123.3, 82.7, 55.0, 39.0, 30.3. Anal. Calcd for $C_{15}H_{20}N_2O_5$: C, 58.4; H, 6.5; N, 9.1. Found: C, 58.4; H, 6.7; N, 9.0.

L-*N*-Boc-aspartic Acid β-Anilide (13b). L-*N*-Boc-aspartic acid anhydride[24] (**1f**) was allowed to react with aniline according to the procedure used for L-*N*-Cbz-glutamic acid γ-anilide (**3b**). Yield: 100% (β:α = 2.2:1). Isolation of the pure β-isomer by flash column chromatography (eluent EtOAc/CH_2Cl_2 1:1, then EtOAc, and then 5% MeOH in EtOAc) gave a white solid. A sample of the product was recrystallized for microanalysis from EtOAc/hexane: mp 153−155 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9H), 2.93 (dd, 1H, J_1 = 16, J_2 = 8 Hz), 3.08−3.20 (m, 1H), 4.47−4.58 (m, 1H), 5.90−5.99 (m, 1H), 7.15−7.22 (m, 1H), 7.30−7.40 (m, 2H), 7.44−7.54 (m, 1H), 7.92 (s, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.6, 172.4, 159.5, 141.4, 131.5, 126.9, 122.9, 82.4, 53.5, 41.4, 30.4. Anal. Calcd for $C_{15}H_{20}N_2O_5$: C, 58.4; H, 6.5; N, 9.1. Found: C, 58.8; H, 6.8; N, 9.1.

Acknowledgment. The authors acknowledge Denis Bilodeau for the elemental analyses reported herein. The authors are also grateful for the financial support of the Université de Montréal, the Natural Sciences and Engineering Research Council of Canada (NSERC), and the Fonds pour la formation de chercheurs et l'aide à la recherche (FCAR) of the province of Québec. One of the authors (Y.R.) also thanks the Région Rhône-Alpes of France for an exchange program bursary.

JO971375E

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