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Unexpected Hydrolytic Instability of N-Acylated Amino Acid Amides and Peptides

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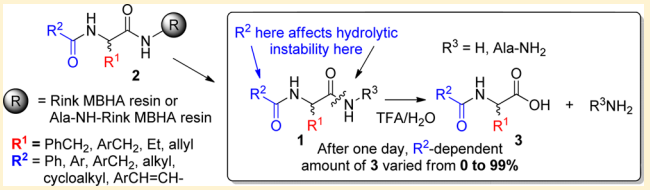
Supporting Information

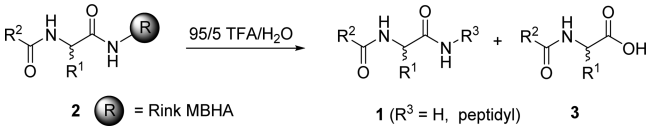
ABSTRACT: Remote amide bonds in simple N-acyl amino acid amide or peptide derivatives **1** can be surprisingly unstable hydrolytically, affording, in solution, variable amounts of **3** under mild acidic conditions, such as trifluoroacetic acid/water mixtures at room temperature. This observation has important implications for the synthesis of this class of compounds, which includes N-terminal-acylated peptides. We describe the factors contributing to this instability and how to predict and control it. The instability is a function of the remote acyl group, R^2CO, four bonds away from the site of hydrolysis. Electron-rich acyl R^2 groups accelerate this reaction. In the case of acyl groups derived from substituted aromatic carboxylic acids, the acceleration is predictable from the substituent's Hammett σ value. N-Acyl dipeptides are also hydrolyzed under typical cleavage conditions. This suggests that unwanted peptide truncation may occur during synthesis or prolonged standing in solution when dipeptides or longer peptides are acylated on the N-terminus with electron-rich aromatic groups. When amide hydrolysis is an undesired secondary reaction, as can be the case in the trifluoroacetic acid-catalyzed cleavage of amino acid amide or peptide derivatives **1** from solid-phase resins, conditions are provided to minimize that hydrolysis.



INTRODUCTION

In preliminary work generalizing our earlier synthesis[1] of N-acylated unnatural amino acid amides **1** to procedures compatible with a wide variety of R^2 groups, an unexpected R^2-dependent hydrolytic instability was encountered (Scheme 1). During the trifluoroacetic acid (TFA) cleavage of the final Rink resin-bound intermediate **2** to give **1**, varying amounts of carboxylic acid **3** were produced.

Scheme 1. Unexpected Presence of Acid 3 in the Cleavage of Resin-Bound Amide 2



It was of concern that the instability of **1** (R^3 = H in the encountered case) could be a more general phenomenon and potentially affect the synthesis of N-acylated natural and unnatural amino acid amides, peptides, and proteins that are targets of organic and peptide chemists. Because of these wider implications, we sought to understand the origins and nature of this instability, to develop predictive tools to anticipate it, and to establish reaction and workup conditions to minimize it.

Amide bonds such as those in N-acylated amino acid amides **1** are generally stable to conditions that would readily cause hydrolysis of an analogous ester bond. Strong acids such as TFA, with varying amounts of water or triethylsilane (TES), are often used to cleave peptides from solid-phase resins without significant hydrolysis of amide bonds.[2] The Rink resin was specifically designed to permit TFA-promoted cleavage of the resin link (such as that in **2**) to provide peptide primary amides **1** (R^3 = H) with both the internal amide(s) and C-terminal primary amide remaining intact.[3]

In general, when amide bond cleavage does occur, it is usually under more vigorous conditions or by virtue of a unique structural feature that permits intramolecular-catalyzed hydrolysis. For example, hydrolysis of a peptide into its constituent amino acids requires 6 N HCl at 100 °C,[4] and cleavage of the N-terminal amino acid from a polypeptide via the classic Edman degradation is promoted by an intramolecular reaction to form a key cyclic intermediate.[5]

Several examples have appeared in the literature in which specific peptidyl amino acid sequences and N-acylated peptides undergo facile amide cleavage under mildly acidic conditions.[6−9] During acid deprotection of a peptide containing

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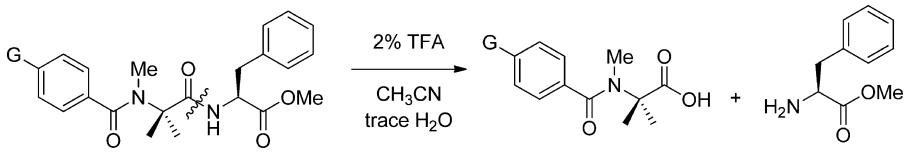
Scheme 2. Hydrolytic Instability of 4 as a Function of the Electronic Nature of G[6]



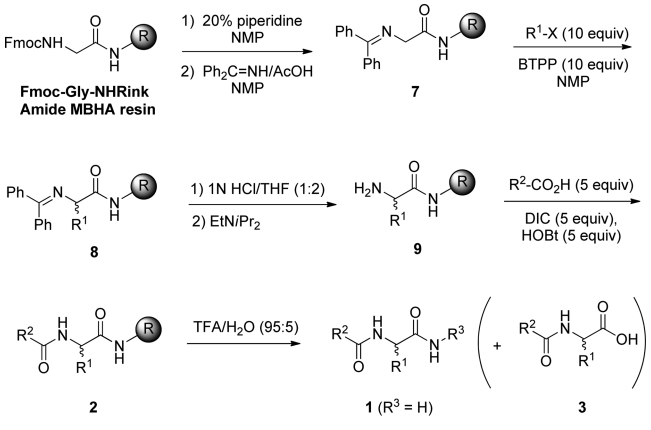
consecutive pipecolic acid residues, Ruzza and co-workers observed unexpected amide bond hydrolysis and provided experimental evidence supporting the intermediacy of an oxazolinium ion.[10] This hydrolytic sensitivity of peptidyl pipecolic acid residues was exploited by Zajdel, Subra, and co-workers in the design of pipecolic resin linkers for solid-phase applications.[11,12]

The closest precedent for the observations reported in this work comes from Goodman and co-workers, who observed amide hydrolysis under mild conditions in a series of *N*-benzoyl-*N*-methylAib-Phe-OMe analogues (**4**; Scheme 2).[6]

It was demonstrated that this instability is a function of the remote benzoyl groups on the N-terminal amino acid of this unusual dipeptide and that the rate of hydrolysis was correlated with the Hammett σ constants of the benzoyl substituents, G. Analysis of the data established the existence of a linear free-energy relationship and supported a mechanism involving the intermediacy of an oxazolinium ion that proceeded from the tertiary amide.

To our knowledge, there are no reports of mild hydrolyses of the simple N-acylated primary amides or peptides represented by **1**. We were surprised, then, in our earlier published work[1] synthesizing **1** (Scheme 3, R^3 = H) from the benzophenone

Scheme 3. Synthesis of N-Acylated Unnatural Amino Acid Amides 1 (R^3 = H)



imine of Rink MBHA glycine amide **7** to observe small amounts of amide bond hydrolysis products **3** under TFA cleavage conditions. This occurred when R^2 was naphthyl but not when it was an Fmoc group.[13a−f]

Our focus in that report was to develop chemistry to incorporate unnatural side chains, R^1, into the α-position of glycine with the acyl group, R^2, being used only to enable isolation and quantification of **1** (R^3 = H) produced in the key alkylation step. Therefore, R^2 was limited to the Fmoc group for all subsequent work quantifying the conditions and success of the alkylation chemistry, and the hydrolysis of the primary amide in the naphthyl case was mentioned only parenthetically.[1]

However, when recently seeking to develop Scheme 3 further into a robust Distributed Drug Discovery (D3)[14−16] lab that would permit the synthesis of large numbers of new compounds through widespread variation of both R^1 and R^2, it became apparent that the amide hydrolysis observed earlier[1] was not an anomaly but a clear function of the nature of the R^2 group and that it would be important to understand the factors involved in this R^2-dependent hydrolytic instability so that its occurrence could be anticipated, predicted, and minimized. This report describes the achievement of that goal.[17]

RESULTS AND DISCUSSION

Creating D3 laboratories involves developing robust, detailed procedures that enable students, in the course of their regular undergraduate organic chemistry lab, to reproducibly synthesize many new biomimetic molecules as potential drug leads for neglected diseases.[14−16] These procedures are often adapted[15,16] from published chemistry.[1,15,18] We were developing a new D3 laboratory, The Combinatorial Synthesis of N-Acylated Unnatural Amino Acid Amides, based on the published reaction sequence shown in Scheme 3.[1] It would be compatible with a wide variety of R^2 groups obtained from carboxylic acids R^2CO_2H. For this adaptation to be successful, it would soon become apparent that understanding and addressing our earlier, parenthetical observation of R^2-dependent hydrolytic instability would be critically important.[1]

This became clear when, in the course of this adaptation, two students conducted independent replicated syntheses of six new molecules, **1a−f** (Scheme 3 and Table 1). The molecules were made through combinatorial reactions carried out in a 2 × 3 combinatorial grid[19] that utilized two different carboxylic acids, R^2CO_2H, in rows A and B and three different alkylating agents, R^1X, in columns 1−3.[14−16]

The replicated and combinatorial nature of this study provided a concise set of compelling observations, revealing an R^2-dependent primary amide instability. If any one of the results for row A (A1−A3) of this grid had been obtained through a single, isolated experiment, then the presence of acid **3** might have been viewed as an anomaly. The fact that every reaction in row A gave significant amounts of carboxylic acids **3a−c** as side products, that none of the reaction mixtures in row B contained hydrolyzed material, and that these results were independently repeated by two researchers suggested the presence of a systematic effect worthy of further inquiry. In addition, the combinatorial nature of these six experiments made it possible to narrow the important variable quickly to the acylating group R^2 (row A vs row B rather than columns 1−3) and to narrow further the common determining variable to the nature of the para substituent on the aromatic acylating agent.

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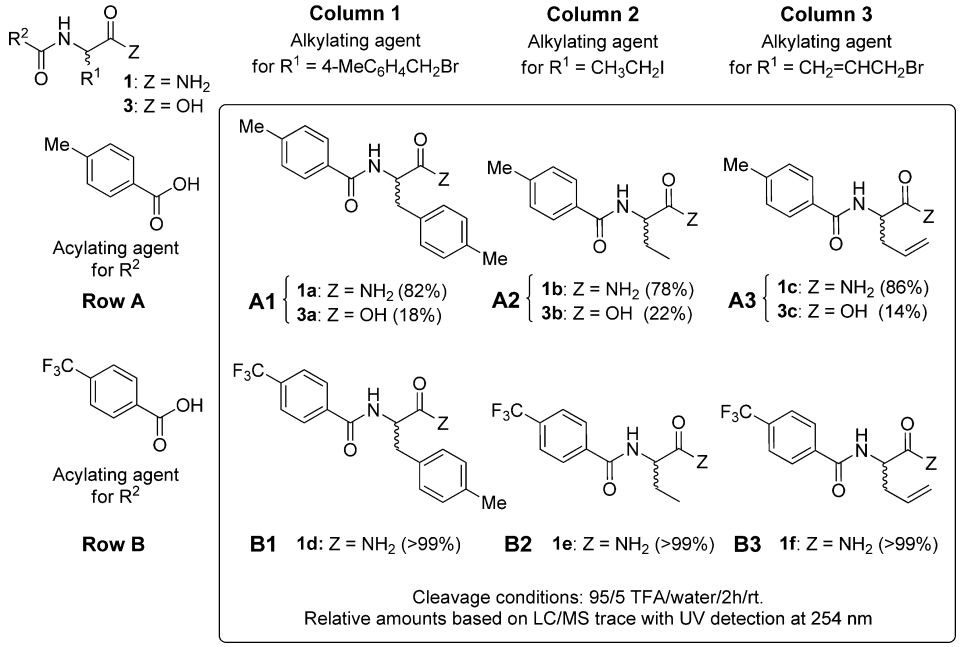
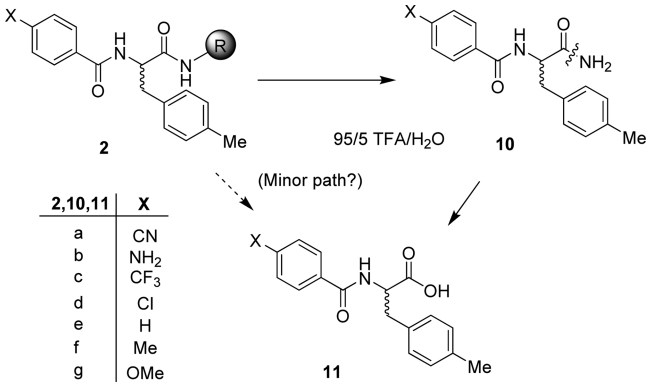
Table 1. Initial Observation of Hydrolytic Instability of 1 (Z = NH_2) as a Function of R^2



On the basis of these observations, it was hypothesized that hydrolysis was a function of the electronic properties of the remote aromatic acyl group, R^2, and that electron-donating groups would facilitate the hydrolysis, whereas electron-withdrawing substituents would suppress it. Accordingly, follow-up experiments were designed to explore this assumption systematically.

Predictive Nature of R^2 Hammett σ Values on Hydrolytic Instability. To undertake a systematic investigation of the R^2-dependent hydrolytic instability, compounds **10a–g**, featuring a representative range of Hammett σ constants[20,21] at the para position of the aromatic benzoyl group, were prepared on Rink Amide MBHA resins (**2a–g**), cleaved, and studied in solution (Scheme 4). After subjection to the original cleavage cocktail (95:5 TFA/water, 0.5 h, rt), all samples were filtered from resin so that subsequent time points (2, 4, 8, and 24 h) would reflect the instability of **10** solely in solution.

Scheme 4. Study of Hydrolysis of 10 (Cleaved from 2) in 95:5 TFA/H_2O



The study was designed to follow the initial formation of hydrolysis products **11a–g** (either directly from **2a–g** or through **10a–g** during the 0.5 h cleavage) along with continuing formation of **11a–g** from **10a–g** in solution at increasing times postcleavage and postfiltration (2, 4, 8, and 24 h). The results from all time points are shown in Table 2 and are reported as the percent of acid **11** present. That the amount of acid increases with time of exposure to the cleavage cocktail after cleavage and filtration from the resin (all time points after 0.5 h) indicates that hydrolysis proceeds primarily in solution and comes directly from **10**.

The data shows that electron-withdrawing substituents (**10a–d**, high σ values) suppress hydrolysis, whereas electron-donating substituents with the lowest Hammett σ values (**10e–g**) are associated with the most rapid hydrolyses to acids. This is readily apparent at all time points and mirrors the results obtained from the acylated *N*-methylAib-Phe-OMe dipeptides studied by Goodman and co-workers (Scheme 2).[6] Plotting the percent acid present versus the Hammett σ constant for the data obtained at 24 h exposure revealed a linear relationship, which allowed qualitative predictions for other substituents to be made (see the Supporting Information).

This study was expanded (Table 3) to include various alkyl groups represented by R^2. Resin precursor **12** was prepared and cleaved to **13**, and time-dependent hydrolysis was studied. The results support those of our initial study: electron donation from R^2 enhances production of N-acylated phenylalanines **14**. Comparison of the results from **13d**, **13e**, **13h**, and **13i** illustrates how hydrolysis increases in proceeding from primary to secondary to tertiary alkyl. The rate-enhancing substituent effect of the *p*-methoxy group can be blocked or retained by carbonyl group insulation (see **13b**: R^2 = 4-MeO-$C_6H_4CH_2$ vs **13j**: R^2 = 4-MeO-C_6H_4) or conjugation (see **13g**: R^2 = 4-MeO-$C_6H_4CH{=}CH$ vs **13j**: R^2 = 4-MeO-C_6H_4), respectively.

Optimization of Cleavage Conditions to Minimize Hydrolysis. Having demonstrated that R^2-dependent amide hydrolysis can occur under typical resin-cleavage conditions, various cleavage methods were investigated to identify conditions that would maximize compound yield while minimizing subsequent hydrolysis. In the earlier study of the Hammett constant-dependent hydrolysis of **10** to **11** (Table 2),

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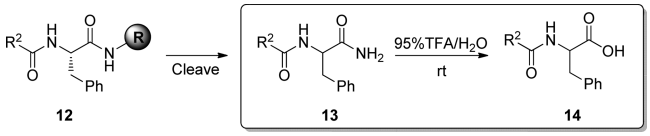
Table 2. Percent of Acid 11 from 10 at Various Exposure Times to 95:5 TFA/H_2O

starting material	X	σ^a	0.5 h[b]	2 h	4 h	8 h	24 h	$t_{1/2}$ (h)[c]
10a	CN	0.66	0	trace	trace	<1	1	nd[d]
10b	NH_2	0.60[e]	trace	trace	trace	1	3	495
10c	CF_3	0.54	0	1	1	3	7	247
10d	Cl	0.23	1	4	7	13	33	42
10e	H	0.00	6	12	18	31	66	16
10f	Me	−0.17	12	26	35	54	90	7.5
10g	OMe	−0.27	19	43	58	80	99	3.8

[a]Linear regression analysis assigns a rho value, ρ, of −2.3 to this reaction; see the Supporting Information. [b]Resin still present at this time point. [c]See the Supporting Information. [d]Not determined. [e]Value for NH_3^+.

Table 3. Percent of Acid 14 Formed from 13 at Various Exposure Times to 95:5 TFA/H_2O



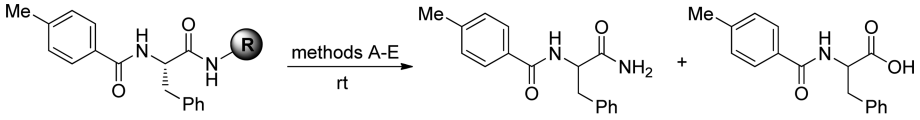
compound **14**	R^2	0.5 h[a]	6 h	23 h
a	9-fluorenylmethyloxy	0	0	0
b	4-MeO-$C_6H_4CH_2$	0	trace	3
c	Me	0	0	3
d	Me_2CHCH_2	3	3	15
e	*c*-propyl	trace	15	59
f	2-naphthyl	3	19	60
g	4-MeO-C_6H_4CH═CH	8	54	80
h	Me_3C	10	40	84
i	1-adamantyl	16	59	92
j	4-MeO-C_6H_4	10	53	96

[a]Resin still present at this time point.

we had already determined that if solutions of **10** were immediately stored at −20 °C after the 0.5 h cleavage time at room temperature then subsequent hydrolysis to **11** was completely suppressed (see the Supporting Information). However, for a robust and uncomplicated D3 procedure, it was necessary to develop optimized cleavage methods that would not require the use of low temperatures. The cleavage of **12k** to **13k** and hydrolysis to **14k** (Table 4) was used as the test case in evaluating a series of cleavage methods. Resin **12k** was prepared and subjected to the varied cleavage conditions. The percent yield of **13k** was determined in each case by quantitative liquid chromatography calibrated with authentic **13k** (QLC, expressed as the percent yield of **13k**), and the ratio of **13k** to hydrolysis product **14k** was determined by proton NMR. Included in the list of methods are mixtures composed of lower percentages of TFA, shorter reaction times, and mixtures in which water was replaced by triethylsilane as a carbocation scavenger.[22] Hydrolysis was substantially reduced (higher **13k**:**14k** ratios) by using cleavage cocktails consisting of 65 or 35% TFA or by elimination of water from the cocktail (entries 3 and 4). However, reducing the percentage of TFA (method E, entries 9 and 10) or reducing the time of cleavage (30 min vs 1 or 2 h) runs the risk of incomplete cleavage from the resin.

The data indicates that reducing cleavage time to 30 min results, without exception, in lower yields of **13k**. The use of 35:60:5 TFA/DCM/water (method D, entries 7 and 8) resulted in the lowest yields, but, interestingly, when triethylsilane was substituted for water (method E, entries 9 and 10), this composition performed significantly better. The improved yields associated with triethylsilane may reflect its ability to irreversibly scavenge the benzhydryl carbocation derived from the resin.[22] Scavenging by water to give the diarylcarbinol would be reversible under the TFA conditions, potentially resulting in reattachment of the cleaved product to

Table 4. Optimization of Cleavage Conditions of 12k as a Function of the Cleavage Method and Time



entry	method[a]	time	pecent yield **13k**[b]	**13k**:**14k**[c]
1	A	30 min	62	86:14
2	A	2 h	76	90:10
3	B	30 min	73	96:4
4	B	1 h	88	95:5
5	C	30 min	55	97:3
6	C	1 h	80	97:3
7	D	30 min	19	96:4
8	D	1 h	32	96:4
9	E	30 min	55	99:1
10	E	1 h	73	98:2

[a]Methods: (A) 95:5 TFA/H_2O; (B) 95:5 TFA/Et_3SiH; (C) 65:30:5 TFA/CH_2Cl_2/Et_3SiH; (D) 35:60:5 TFA/CH_2Cl_2/H_2O; and (E) 35:60:5 TFA/CH_2Cl_2/Et_3SiH. [b]Measured by QLC. [c]Measured by ^1H NMR.

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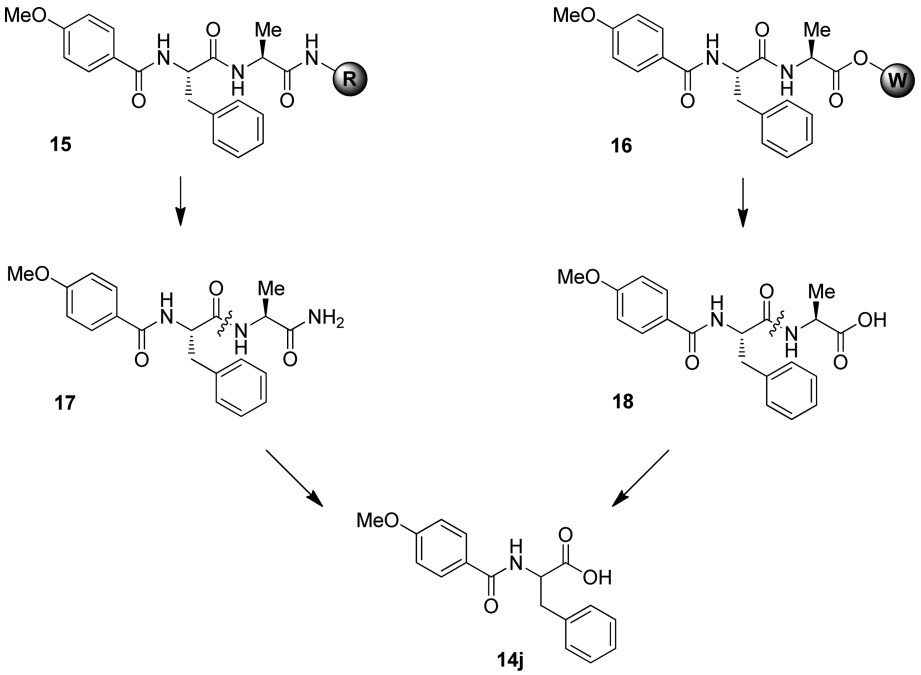
Scheme 5. Instability of N-Acylated Peptides as a Function of the Acyl Group



the resin via the primary amide or by way of Friedel–Crafts alkylation of the aromatic ring.[23] The combination of a 1 h cleavage, replacement of water with triethylsilane, and use of a lower percentage of TFA represents the preferred methods that are described by C and E (entries 6 and 10).

R^2-Dependent N-Acylated Peptide Instability. Our observation and analysis of the mild hydrolysis promoted by remote aromatic acyl groups R^2 on the N-terminus of simple N-acylated amino acid amides **1** (R^3 = H) prompted an additional experiment documenting its implications for N-acylated peptide instability. Rink Amide MBHA and Wang N-acylated dipeptide resins **15** and **16**, respectively, were prepared and subjected to cleavage (95:5 TFA/water, 0.5 h, rt, Scheme 5). Analysis by LC/MS was performed after 0.5, 6, and 24 h (Table 5).

Table 5. Ratio of Dipeptide 17 or 18 to Hydrolysis Product 14j at Various Times

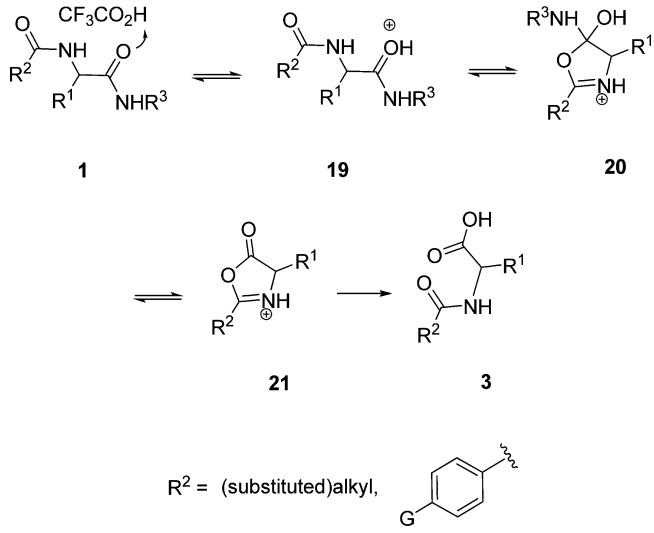
source of **14j**	0.5 h[a]	6 h	24 h
17:**14j** (from Rink resin)	~99:1	89:11	60:40
18:**14j** (from Wang resin)	~99:1	88:12	49:51

[a]Resin still present at this time point.

Although negligible hydrolysis occurred in the initial 30 min exposure, by 6 h, 10–15% of the resin-free mixture was composed of hydrolysis product **14j**,[24] increasing to nearly 50% by 24 h.

Mechanistic Considerations. Observations from the above studies are consistent with hydrolysis proceeding through a rate-limiting step in which a positively charged transition state is stabilized by an electron-donating R^2 group. A proposed mechanism[25,26] is depicted in Scheme 6. Initial protonation at the C-terminal amide carbonyl[27] to give **19** is followed by cyclization to the tetrahedral intermediate **20**. Collapse of the tetrahedral intermediate affords an oxazolinium ion (**21**) of the type originally proposed by Urban et al.[7] and subsequently put forward by others.[6,10,28,29] Hammett substituent effects arising

Scheme 6. Proposed Mechanism for Formation of Hydrolysis Products 3



from R^2 (alkyl or aryl) might be expected to be operative in the cyclization of **19** to **20** or in the acid-catalyzed ring opening of **21** to **3**.[30]

Possible Utility of N-Acyl Group-Dependent Lability. In the future, the substituent-dependent amide hydrolytic lability we have observed could be advantageously used. With the appropriate choice of the aryl group, a peptide temporarily acylated with ArCO-AA-OH (Ar = electron-rich aryl group, AA = amino acid residue) could be converted back to the unacylated parent peptide under mild, perhaps even physiological, conditions. For example, if the peptide's physical properties require modification to improve its pharmacological profile, then a highly electron-rich Ar group might permit ArCO-AA-OH to be temporarily attached and then removed in a prodrug strategy.[31,32]

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CONCLUSIONS

This report shows that amide bonds in simple N-acylated amino acid amide or peptide derivatives can be surprisingly unstable. When an electron-rich aromatic carboxylic acid is acylated to the amino nitrogen of a simple amino acid carboxamide or the N-terminus of a peptide, room-temperature trifluoroacetic acid/water mixtures result in hydrolysis of the amide bond four bonds away from the NH-acyl group. Even simple aromatic acyl derivatives, when appropriately substituted with electron-donating substituents, can promote facile remote amide cleavage. In particular, this hydrolysis has been found to be sensitive to the substituent effects of R^2 located on a remote N-terminal aromatic acyl group. In the case of N-benzoyl amides, the relative rate of hydrolysis can be predicted from the substituent's Hammett σ value. After 24 h at room temperature, compounds having benzoyl acyl substituents with negative σ values are almost completely hydrolyzed, those with values between 0 and 0.60 are moderately hydrolyzed, and those with values $\geq$0.60 show minimal amide cleavage. Acyl groups derived from secondary or tertiary alkyls are electron-rich and also promote substantial hydrolysis after 6 h at room temperature.

This acyl group-dependent acid-catalyzed lability occurs under acidic conditions commonly employed when cleaving products from solid-phase resins. When these products are N-acylated peptides, the hydrolysis reaction could become problematic. To address this issue, a systematic evaluation of cleavage conditions was conducted. It was found that even with hydrolysis-promoting substituents postcleavage hydrolysis can be minimized by the use of cleavage cocktails containing 65% or less TFA, replacing water with triethylsilane.

It is important to note that in the current study N-capped dipeptides are also hydrolyzed under typical cleavage conditions. This suggests that unwanted peptide truncation may occur during synthesis or storage when dipeptides or longer peptides are acylated with electron-rich aromatic groups on the N-terminus. The combined ability to predict hydrolytic instability based on acyl group Hammett σ values with the availability of cleavage conditions to minimize the predicted hydrolysis should help chemists working in this field to design appropriate synthetic strategies to N-acylated amino acid amides and peptides.

EXPERIMENTAL SECTION

General Methods. All reagents were commercially available and were used without further purification. Fmoc-protected amino acid-bound Rink Amide MBHA and Wang resins were purchased from Peptides International (Louisville, KY). Boc-protected amino acid-bound Merrifield resins were purchased from Novabiochem (EMD Millipore) or Polymer Laboratories (now Agilent). Combinatorial and parallel synthetic sequences and resin cleavage/hydrolysis experiments were performed in custom-made 3.5 mL glass reaction vessels containing a sintered glass frit and screw-cap ends fitted with poly(tetrafluoroethylene) (PTFE) septa all purchased from Chemglass (Vineland, NJ). The reaction vessels were secured in polypropylene Bill-Boards consisting of a 2 × 3 or 4 × 6 array of position holes fitted with rubber O-rings and were supplied by Leads Metal (Indianapolis, IN). Synthetic sequences for the preparation of advanced intermediate resins were performed in 25 or 50 mL solid-phase peptide synthesis vessels (SPPS) purchased from Chemglass. Rotation of Bill-Boards was accomplished using a spit rod fitted with three 6-brackett metal assemblies custom-made by Leads Metal and was driven by a Jenn-Air rotisserie motor or using brackets to fit 2 × 3 or 4 × 6 Bill-Boards held in a rotovap assembly.

General Procedure for the Preparation and Distribution of Isopycnic Suspensions of Resins. *Preparation.* Approximately 80−100 mL of isopycnic solvent per gram of resin was used. To the resin contained in a 150 mL beaker with stirrer bar was added 60 mL of NMP followed by 30 mL of DCM. The mixture was briefly stirred and was then allowed to stand. If resin showed a tendency to accumulate downward, then DCM was added until there was no overall movement upward or downward. If resin showed a tendency to rise, then NMP was added until neutral buoyancy was achieved.

Distribution. Distribution of equal quantities of resin to multiple 3.5 mL glass reaction vessels in a Bill-Board was accomplished with the use of an Eppendorf repeater pipet fitted with a 50 mL tip. An aliquot of 2 mL was dispensed to each vessel followed by an aliquot of 1 mL to each vessel. This procedure was repeated until the number of milliliters of isopycnic suspension remaining in the beaker was judged to be less than the number of vessels, n (the smallest volume needed to dispense 1 mL into all vessels in a single pass). Isopycnic solvent was added to the beaker to give a volume of $(2n + 10)$ mL. A 2 mL aliquot was dispensed to each vessel. Isopycnic solvent was added to the beaker again to give a volume of $(2n + 10)$ mL. Aliquots of 2 mL were distributed to each vessel again. The resin in each vessel was then washed with 4 × 2 mL of the solvent to be used in the next step.

General Procedure of Resin Cleavage. This procedure is associated with the results presented in Tables 1 and 4. The DCM-washed resins, contained in 3.5 mL reaction vessels, were treated with 1.5 to 2 mL of cleavage reagent. The vessels were rotated at room temperature for the required time period (0.5, 1, or 2 h) and were then drained into 5-dram, tooled-neck vials. Each resin was then washed with 2 mL of the cleavage cocktail followed by 2 mL of DCM. Aliquots of 100 μL were removed and immediately evaporated to dryness under a stream of nitrogen, and the residue was dissolved in acetonitrile for LC/MS analysis.

LC/MS Analyses of Products. Analyses were performed using an Agilent Eclipse XDB-C18 5 μm column, 4.6 × 150 mm length, with 5 μL injections and a flow rate of 1.0 mL/min. A linear gradient from 20% 1:1 MeCN/MeOH (5 mM NH_4OAc) and 80% water (5 mM NH_4OAc) to 100% 1:1 MeCN/MeOH (5 mM NH_4OAc) over 10 min was used. Detection was performed at 254, 214, and 210 nm. Percent amide and/or percent acid were calculated from the reported peak areas observed at 254 nm unless otherwise noted.

Combinatorial Preparation of Resins 2 (Scheme 3, R^1 = 4-Methylbenzyl, Ethyl, or Allyl; R^2 = 4-Methylphenyl or 4-Trifluoromethylphenyl). These resins were prepared for the cleavage study reported in Table 1. A 25 mL SPPS vessel was charged with 1.36 g (0.598 mmol, 0.44 mmol/g) of Fmoc-Gly-NH-Rink MBHA resin. The resin was washed with 3 × 15 mL of NMP and was then treated with 5 × 10 mL washes of 20% (v/v) piperidine in NMP delivered over a 35 min period. The deprotected resin was then washed with 3 × 15 mL of NMP and was treated with 1.09 g (6.01 mmol, 10.0 equiv) of benzophenone imine in 9 mL of NMP followed by 0.313 g (5.22 mmol, 8.72 equiv) of acetic acid in 1 mL of NMP. The vessel was rocked on an orbital shaker for 22 h and then drained, and the resin was washed with 3 × 10 mL each of NMP, THF, 3:1 THF/H_2O, THF, and DCM to give resin **7**. The resin was transferred to a 150 mL beaker using NMP, was made into an isopycnic suspension, and was equally distributed to twelve 3.5 mL reaction vessels contained in two 2 × 3 Bill-Boards to provide 50 μmol to each vessel (see the General Procedure for the Preparation and Distribution of Isopycnic Suspensions of Resins). The resins were then washed with 3 × 2 mL of NMP. Each Bill-Board was then treated identically as follows: Phosphazene base *tert*-butylimino-tri(pyrrolidino)-phosphorane (BTPP, 1.0 M, 0.50 mL, 0.50 mmol, 10 equiv) in NMP was added to each vessel and allowed to stand for 5 min. 4-Methylbenzyl bromide, ethyl iodide, and allyl bromide (0.50 mL of 1.0 M solutions in NMP, 0.50 mmol, 10 equiv) were added, respectively, to column 1 vessels A1/B1, column 2 vessels A2/B2, and column 3 vessels A3/B3. The Bill-Boards were rotated for 22 h, the vessels were drained, and the resins were washed with 3 × 2 mL each of NMP, DCM, and THF to give resins **8** (Scheme 3, R^1 = 4-methylbenzyl, ethyl, or allyl). To each vessel was added 2 mL of 1.0 N HCl/THF

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(1:2), and the Bill-Boards were rotated for 20 min, the vessels were drained, and the resins were washed with 3 mL of THF, 2 × 2.5 mL of 0.2 M diisopropylethylamine in NMP, and 2 × 2.5 mL of NMP to give resins **9** (Scheme 3, R^1 = 4-methylbenzyl, ethyl, allyl). 4-Methylbenzoic acid and 4-trifluoromethylbenzoic acid (0.25 M, 1.0 mL, 0.25 mmol, 5.0 equiv) in 0.25 M 1-hydroxybenzotriazole (HOBt) in NMP were added, respectively, to row A vessels A1−A3 and row B vessels B1−B3. Each vessel was then treated with 0.50 mL (0.25 mmol, 5.0 equiv) of 0.50 M diisopropylcarbodiimide in NMP, and the Bill-Boards were rotated for 22 h. The vessels were drained, and the resins were washed with 2 × 3 mL each of NMP, THF, and 3 × 3 mL of DCM to afford resins **2** (Scheme 3). Cleavage of each resin with 95:5 TFA/water for 2 h was performed as described above (General Procedure of Resin Cleavage).

LC/MS and ^{1}H NMR Results of TFA-Mediated Cleavage of 2 × 3 Combinatorial Bill-Boards (Scheme 3 and Table 1). Cleavage of **2** gave the crude products in Table 1 (see General Procedure of Resin Cleavage). Analytical data for crude products **1** (Z = NH_2) and **3** (Z = OH) include LC/MS and diagnostic peaks in the ^{1}H NMR.

***α*-[(4-Methylbenzoyl)amino]-4-methylbenzenepropanamide (1a) and *N*-(4-Methylbenzoyl)-4-methylphenylalanine (3a).** For **1a**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.30 (s), 2.38 (s), 3.19 (dd), 3.29 (dd), 5.01 (dd), 7.55 (d); LC/MS (ESI): 8.70 min, m/z 297 $[M + H]^+$. For **3a**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 3.13 (2dd), 4.94 (dd), 7.59 (d); LC/MS (ESI): 6.36 min, m/z 298 $[M + H]^+$.

***N*-(2-Amino-1-ethyl-2-oxoethyl)-4-methylbenzamide (1b) and 2-[(4-Methylbenzoyl)amino]butanoic Acid (3b).** For **1b**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.02 (t), 1.90 (septet), 2.08 (septet), 4.75 (dd), 7.68 (d); LC/MS (ESI): 6.08 min, m/z 221 $[M + H]^+$. For **3b**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.83 (septet), 1.99 (septet), 4.68 (dd); LC/MS (ESI): 3.64 min, m/z 222 $[M + H]^+$.

***N*-[(1-Aminocarbonyl)-3-buten-1-yl]-4-methylbenzamide (1c) and 2-[(4-Methylbenzoyl)amino]-4-pentenoic Acid (3c).** For **1c**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.40 (s), 2.60−2.78 (m), 4.81 (dd), 5.18−5.23 (m), 5.74−5.82 (m), 7.24 (d), 7.65 (d); LC/MS (ESI): 6.66 min, m/z 233 $[M + H]^+$. For **3c**: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 4.77 (dd); LC/MS (ESI): 4.02 min, m/z 234 $[M + H]^+$.

***α*-[(4-Trifluoromethylbenzoyl)amino]-4-methylbenzenepropanamide (1d).** ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.32 (s), 3.10 (dd), 3.19 (dd), 4.85 (m), 7.70 (d), 7.86 (d); LC/MS (ESI): 9.39 min, m/z 351 $[M + H]^+$.

***N*-(2-Amino-1-ethyl-2-oxoethyl)-4-trifluoromethylbenzamide (1e).** ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.02 (t), 1.80 (septet), 2.02 (septet), 4.62 (m), 7.72 (d), 7.93 (d); LC/MS (ESI): 7.39 min, m/z 275 $[M + H]^+$.

***N*-[(1-Aminocarbonyl)-3-buten-1-yl]-4-trifluoromethylbenzamide (1f).** ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.64 (m), 4.74 (m), 5.19−5.23 (m), 5.78−5.85 (m), 7.71 (d), 7.90 (d); LC/MS (ESI): 7.85 min, m/z 287 $[M + H]^+$.

Preparation of Resins 2a−g from Resin 7 (Scheme 3). *Preparation of Resin **7** from Fmoc-Gly-NH-Rink MBHA Resin.* A 50 mL SPPS vessel was charged with 3.43 g (1.75 mmol, 0.51 mmol/g) of Fmoc-Gly-NH-Rink MBHA resin. The resin was washed with 3 × 15 mL of NMP and was then treated with 5 × 20 mL washes of 20% (v/v) piperidine in NMP delivered over a 45 min period. The deprotected resin was then washed with 4 × 15 mL of NMP and was treated with 3.16 g (17.4 mmol, 9.94 equiv) of benzophenone imine in 15 mL of NMP followed by 910 mg (15.2 mmol, 8.70 equiv) of acetic acid. The vessel was rocked on an orbital shaker for 20 h and then drained, and the resin was washed with 3 × 20 mL each of NMP, THF, 3:1 THF/H_2O, THF, and DCM. Resin **7** was dried under a gentle stream of nitrogen gas for 2 h and then under vacuum for 3 h.

*Preparation of Resin **8** (R^1 = 4-Methylbenzyl).* Resin **7** (1.75 mmol) was swelled in the SPPS vessel with 30 mL of NMP for 30 min. The vessel was drained, and the resin was treated with 5.49 g (17.6 mmol, 10.0 equiv) of phosphazene base *tert*-butylimino-tri(pyrrolidino)phosphorane (BTPP) in 9.5 mL of NMP. The contents were gently agitated by hand to affect mixing, and the mixture was treated with 3.26 g (17.6 mmol, 10.0 equiv) of 4-methylbenzyl bromide in 14.3 mL of NMP. The vessel was rocked overnight for 24 h and then drained, and the resin was washed with 3 × 20 mL each of NMP, DCM, and THF. Resin **8** was dried under a slow stream of argon for 45 min and was then stored overnight.

*Preparation of Resin **9** (R^1 = 4-Methylbenzyl) and Distribution to 3.5 mL Reaction Vessels.* Resin **8** was treated with 30 mL of 2:1 THF/1.0 N HCl, and the vessel was rocked for 40 min and then drained. The resin was washed with 3 × 20 mL of THF, neutralized with 3 × 20 mL washes of 0.2 M diisopropylethylamine (DIEA) in NMP, and then washed with 3 × 20 mL of NMP to give resin **9**. Resin **9** was then quantitatively transferred to a 400 mL beaker using 100 mL of NMP to facilitate the transfer. Dichloromethane (50 mL) was then added, and the composition was adjusted to achieve neutral buoyancy. A final volume of 200−250 mL was then equally dispensed 2 mL at a time into a 5 column × 7 row array of reaction vessels with uncapped bottoms held in two 4 × 6 Bill-Boards (see General Procedure for the Preparation and Distribution of Isopycnic Suspensions of Resins). Each vessel was then washed with 3 × 2 mL of NMP and then capped.

*Preparation of Resins **2a−g** (R^1 = 4-Methylbenzyl).* The array of 35 vessels each containing 50.0 μmol of resin **9** was treated in the following manner: 1.0 mL of a 0.25 M solution of row 1 carboxylic acid $^1RCO_2H$ (250 μmol, 5.0 equiv) dissolved in 0.25 M 1-hydroxybenzotriazole (250 μmol, 5.0 equiv, HOBt) in NMP was added to each of the five vessels in row 1 followed by 0.5 mL of a 0.50 M solution of diisopropylcarbodiimide (250 μmol, 5.0 equiv, DIC) in NMP. The five vessels in each of the remaining six rows were treated in an identical manner with its assigned carboxylic acid, HOBt, and DIC. The Bill-Board was rotated for 2 days, and the vessels were drained and then washed with 3 × 1.5 mL each of NMP, THF, and DCM to give resins **2a−g**.

TFA-Mediated Cleavage of Resins 2a−g to 10a−g and 11a−g (Scheme 4, Hammett Study, Table 2). Each of the 35 vessels in the 5 × 7 array (50 μmol each) was treated with 1.5 mL of 95:5 TFA/H_2O and was rotated at room temperature for 30 min. All vessels were then drained into collection vials, and the resins were washed with 1 mL of 95:5 TFA/H_2O. A 90 μL aliquot from the combined filtrates of each acylated amide **10a−g** in column 1 (representing the 0.5 h exposure) was removed and immediately evaporated to dryness under a stream of nitrogen. These samples were diluted with acetonitrile and analyzed by LC/MS. The remainder of each filtrate in column 1 (0.5 h exposure) was then stored at −20 °C and assayed by LC/MS again after 48 h. These column 1 filtrates, stored at −20 °C, would be worked up for the purpose of isolating primary amides **10a−g**. Filtrates in columns 2−5 were allowed to stand at room temperature for 1.5, 3.5, 7.5, and 23.5 h, respectively, and were then assayed by LC/MS (Table 2). After sampling for analysis, filtrates were stored at −20 °C. Complete conversion of amides **10e−g** to acids **11e−g** (X = H, Me, and OMe), for the purpose of isolation and characterization, was accomplished by allowing their column 5 filtrates to stand at room temperature for 5 days.

Isolation of Amides 10a−g. After storage at −20 °C for 2 days, each solution from column 1 was added to 100 mL of cold 1.0 N sodium hydroxide. The mixture was then extracted with two 20 mL portions of DCM. The combined extracts were dried over Na_2SO_4 and were concentrated to give crude amides **10a−g** as amorphous solids.

***α*-[(4-Cyanobenzoyl)amino]-4-methylbenzenepropanamide (10a).** Yield 7.6 mg (49%), mp 235−238 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.93 (dd, J = 13.6 and 10.9 Hz, 1H), 3.09 (dd, J = 13.7 and 3.9 Hz, 1H), 4.62 (ddd, J = 10.8, 8.5, and 4.1 Hz, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.13 (br s, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.61 (br s, 1H), 7.94 (s, 4H), 8.80 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.7, 55.0, 113.5, 118.2, 128.1, 128.6, 128.9, 132.2, 135.0, 135.2, 138.0, 164.7, 173.0; HRMS (ESI-TOF): m/z $[M + Na]^+$ calcd for $C_{18}H_{17}N_3O_2Na$, 330.1219; found, 330.1215.

***α*-[(4-Aminobenzoyl)amino]-4-methylbenzenepropanamide (10b).** Yield 4.8 mg (32%), mp 208−211 °C (MeOH); ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.92 (dd, J = 13.6 and 10.4 Hz, 1H), 3.01 (dd, J = 13.7 and 4.2 Hz, 1H), 4.53 (ddd, J = 10.2, 8.5, and 4.2 Hz, 1H), 5.60 (br s, 2H), 6.51 (d, J = 8.6 Hz, 2H), 7.02 (br s,

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1H), 7.04 (d, J = 7.9 Hz, 2H), 7.18 (d, J = 8.0 Hz, 2H), 7.41 (br s, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.90 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.8, 54.5, 112.3, 120.6, 128.5, 128.8, 128.9, 134.8, 135.5, 151.6, 165.9, 173.7; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{17}H_{19}N_3O_2Na$, 320.1375; found, 320.1386.

α-[(4-Trifluoromethylbenzoyl)amino]-4-methylbenzenepropanamide (10c). Yield 4.1 mg (23%), mp 220−224 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.94 (dd, J = 13.6 and 10.9 Hz, 1H), 3.09 (dd, J = 13.7 and 3.9 Hz, 1H), 4.63 (ddd, J = 10.9, 8.2, and 4.1 Hz, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.12 (br s, 1H), 7.22 (d, J = 7.9 Hz, 2H), 7.58 (br s, 1H), 7.83 (d, J = 8.3 Hz, 2H), 7.99 (d, J = 8.1 Hz, 2H), 8.75 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.7, 54.9, 123.8 (q, $^1J_{CF}$ = 272.4 Hz), 125.1 (q, $^3J_{CF}$ = 3.6 Hz), 128.2, 128.5, 128.9, 131.1 (q, $^2J_{CF}$ = 31.7 Hz), 135.0, 135.2, 137.8, 164.9, 173.0; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{18}H_{18}F_3N_2O_2$, 351.1315; found, 351.1317.

α-[(4-Chlorobenzoyl)amino]-4-methylbenzenepropanamide (10d). Yield 7.5 mg (47%), 225.5−227 °C (EtOH); ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.92 (dd, J = 13.7 and 10.8 Hz, 1H), 3.06 (dd, J = 13.7 and 4.0 Hz, 1H), 4.59 (ddd, J = 10.8, 8.4, and 4.1 Hz, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.10 (br s, 1H), 7.20 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.56 (br s, 1H), 7.82 (d, J = 8.7 Hz, 2H), 8.58 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.7, 54.8, 128.1, 128.5, 128.9, 129.2, 132.8, 135.0, 135.3, 135.9, 165.0, 173.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{18}ClN_2O_2$, 317.1051; found, 317.1050.

α-(Benzoylamino)-4-methylbenzenepropanamide (10e).[33] Yield 8.2 mg (58%), mp 216−220 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.22 (s, 3H), 2.93 (dd, J = 13.6 and 10.6 Hz, 1H), 3.06 (dd, J = 13.7 and 4.1 Hz, 1H), 4.61 (ddd, J = 10.6, 8.4, and 4.1 Hz, 1H), 7.05 (d, J = 7.8 Hz, 2H), 7.10 (br s, 1H), 7.22 (d, J = 8.0 Hz, 2H), 7.44 (t, J = 7.5 Hz, 2H), 7.51 (tt, J = 7.4 and 1.3 Hz, 1H), 7.54 (br s, 1H), 7.80 (d, J = 7.1 Hz, 2H), 8.46 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.7, 54.8, 127.3, 128.0, 128.5, 128.9, 131.1, 134.0, 134.9, 135.3, 166.0, 173.3; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{19}N_2O_2$, 283.1441; found, 283.1440.

α-[(4-Methylbenzoyl)amino]-4-methylbenzenepropanamide (10f). Yield 8.0 mg (54%), mp 203−206 °C; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.32 (s, 3H), 2.40 (s, 3H), 3.07 (dd, J = 13.9 and 8.0 Hz, 1H), 3.24 (dd, J = 13.8 and 5.8 Hz, 1H), 4.83 (m, 1H), 5.32 (br s, 1H), 5.73 (br s, 1H), 6.77 (d, J = 7.1 Hz, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 8.0 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.6, 20.9, 36.8, 54.8, 127.4, 128.58, 128.60, 129.0, 131.3, 135.0, 135.4, 141.0, 165.9, 173.4; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{18}H_{21}N_2O_2$, 297.1598; found, 297.1595.

α-[(4-Methoxybenzoyl)amino]-4-methylbenzenepropanamide (10g). Yield 6.6 mg (42%), mp 210−212 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.21 (s, 3H), 2.93 (dd, J = 13.6 and 10.6 Hz, 1H), 3.03 (dd, J = 13.7 and 4.0 Hz, 1H), 3.70 (s, 3H), 4.57 (ddd, J = 10.5, 8.4, and 4.1 Hz, 1H), 6.97 (d, J = 7.8 Hz, 2H), 7.04 (d, J = 7.9 Hz, 2H), 7.06 (br s, 1H), 7.21 (d, J = 8.0 Hz, 2H), 7.49 (br s, 1H), 7.79 (d, J = 8.9 Hz, 2H), 8.29 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.5, 36.7, 54.7, 55.2, 113.2, 126.2, 128.5, 128.9, 129.1, 134.9, 135.4, 161.4, 165.4, 173.4; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{18}H_{20}N_2O_3Na$, 335.1372; found, 335.1362.

Isolation and Purification of Acids 11e−g (Hammett Study, Scheme 4). The benzoyl substituents present in the corresponding amides **10e−g** facilitate hydrolysis at rates high enough to result in complete conversion to the acids over time, rendering convenient isolation and purification.

***N*-Benzoyl-4-methylphenylalanine (11e).**[34] After standing at room temperature for 5 additional days, the filtrate from the 24 h postcleavage exposure was evaporated under a stream of nitrogen gas to give 14.4 mg of a residue that was chromatographed on a prepacked 500 mg column of Hypersep CN (cyanosilica, Thermo Scientific) using step-gradient elution with hexanes (3 × 3 mL), 8:2 hexanes/acetone (3 mL), and eluting the desired material with 7:3 hexanes/acetone to give 6.9 mg (51%) **11e** as an amorphous solid, mp 171−172 °C; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 2.32 (s, 3H), 3.23 (dd, J = 14.1 and 6.0 Hz, 1H), 3.32 (dd, J = 14.1 and 5.6 Hz, 1H), 5.03 (m, 1H), 6.51 (br d, J = 7.0 Hz, 1H), 7.11 (m, 4H), 7.42 (t, J = 7.6 Hz, 2H), 7.52 (t, J = 7.4 Hz, 1H), 7.68 (d, J = 7.9 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 21.1, 37.8, 55.7, 128.4, 129.5, 130.1, 130.2, 132.8, 135.4, 135.6, 137.5, 170.2, 174.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{18}NO_3$, 284.1281; found, 284.1285.

***N*-[(4-Methyl)benzoyl]-4-methylphenylalanine (11f).** After standing at room temperature for 5 additional days, the filtrate from the 24 h postcleavage exposure was evaporated under a stream of nitrogen gas to give 16.5 mg of a residue that was chromatographed on a Dynamax Microsorb C-18, 5 μm column (21.4 × 250 mm) using the isochratic system of 50/50 1:1 MeCN/MeOH (5 mM ammonium acetate)/water (5 mM ammonium acetate) at a 5 mL/min flow rate to give 8.6 mg (61%) **11f** as an amorphous solid after evaporation, mp 184−187 °C; ^{1}H NMR (500.13 MHz, CD_3OD): δ 2.26 (s, 3H), 2.37 (s, 3H), 3.07 (dd, J = 13.8 and 8.3 Hz, 1H), 3.27 (dd, J = 13.8 and 4.9 Hz, 1H), 4.75 (dd, J = 8.2 and 5.0 Hz, 1H), 7.05 (d, J = 7.9 Hz, 2H), 7.12 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 8.0 Hz, 2H), 7.61 (d, J = 8.2 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 21.1, 21.4, 38.2, 56.5, 128.3, 129.9, 130.1, 130.3, 132.7, 135.9, 137.2, 143.4, 169.7, 176.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{18}H_{20}NO_3$, 298.1438; found, 298.1441.

***N*-[(4-Methyoxy)benzoyl]-4-methylphenylalanine (11g).** After standing an additional 3 days at −20 °C, the filtrate from the 24 h postcleavage exposure was evaporated under a stream of nitrogen gas to give 17.1 mg of a residue that was chromatographed on a prepacked 500 mg column of Hypersep CN (cyanosilica, Thermo Scientific) using a step-gradient elution with hexanes (3 mL), 8:2 hexanes/acetone (3 mL), and eluting the desired material with 7:3 hexanes/acetone to give 5.3 mg (36%) **11g** as an amorphous solid, mp 168−72 °C; ^{1}H NMR (500.13 MHz, CD_3OD): δ 2.27 (s, 3H), 3.07 (dd, J = 13.9 and 9.3 Hz, 1H), 3.27 (dd, J = 13.9 and 5.0 Hz, 1H), 3.83 (s, 3H), 4.80 (dd, J = 9.4 and 5.1 Hz, 1H), 6.94 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 7.9 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 7.71 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 21.1, 37.8, 55.7, 55.9, 114.7, 127.4, 130.1, 130.2, 130.3, 135.6, 137.4, 164.1, 169.7, 175.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{18}H_{20}NO_4$, 314.1387; found, 314.1390.

Preparation of *N*-Acylated (*S*)-Phenylalanine-NH-Rink Amide MBHA Resins 12b−j. A 50 mL SPPS vessel was charged with 780−940 mg (250−300 μmol) of Fmoc-Phe-NH-Rink Amide MBHA resin (0.32 mmol/g, Peptides International) and treated with 20 mL of 20% (v/v) piperidine in *N*-methylpyrolidinone (NMP). The mixture was rocked for 45 min at room temperature and was then drained. The resin was washed with 4 × 15 mL of NMP and was then diluted with 55:45 NMP/DCM to make an isopycnic mixture (neutral buoyancy). The mixture was then equally distributed to 3.5 mL reaction vessels using a repeater pipet (see General Procedure for the Preparation and Distribution of Isopycnic Suspensions of Resins). Each resin was washed with 4 × 1.5 mL of NMP and was then treated with a 0.25 M solution of the required carboxylic acid (5.00 equiv) in 0.25 M *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (5.00 equivalents, HBTU) in DMF followed by 10.0 equiv of a 0.50 M solution of diisopropylethylamine in DMF. The vessels were rotated at room temperature for 48 h and were then drained. Each resin was washed with 5 × 1.5 mL of DMF and 6 × 1.5 mL of DCM and was then stored overnight in its capped vessel.

TFA-Mediated Cleavage of Resins 12a−j to 13 and 14 (Table 3). Each vessel containing 50 μmol of resin **12a−j** was treated with 1.5 mL of 95:5 TFA/H_2O and was rotated at room temperature for 0.5 h. All vessels were then drained into collection vials, and the resins were washed with 1.5 mL of 95:5 TFA/H_2O followed by 1.5 mL of DCM. Three 200 μL aliquots from each vial were removed and placed in sets of three vials representing the three time exposures of 0.5, 6, and 23 h, at which point they were evaporated under a stream of nitrogen gas and analyzed by LC/MS (214 nm) as solutions in acetonitrile. To prevent further hydrolysis of primary amides **13** after 30 min exposure to the TFA solution, the vials containing the bulk of the filtrates were stored at −20 °C.

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9*H*-Fluoren-9-ylmethyl *N*-[2-Amino-2-oxo-1-(phenylmethyl)ethyl]carbamate [Fmoc-Phe-NH_2] (13a).[35] The filtrate was evaporated to dryness under a stream of nitrogen to give 6.6 mg of crude material. Trituration under diethyl ether afforded 6.1 mg (33%) of **13a** as an amorphous solid, mp 192−194 °C (dec); ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.78 (dd, J = 13.5 and 10.6 Hz, 1H), 3.00 (dd, J = 13.6 and 4.0 Hz, 1H), 4.16−4.19 (m, 4H), 7.08 (br s, 1H), 7.18 (t, J = 7.1 Hz, 1H), 7.24−7.33 (m, 6H), 7.39−7.43 (m, 2H), 7.45 (br s, 1H), 7.53 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.88 (d, J = 7.5 Hz, 2H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 37.4, 46.4, 55.9, 65.5, 120.0, 125.17, 125.25, 126.1, 126.9, 127.5, 127.9, 129.1, 138.2, 140.5, 143.6, 143.7, 155.7, 173.3 (Note that two extra aromatic signals are present, indicative of the diastereotopic nature of the aromatic carbons, which display identical atom connectivity. Two extra signals are also present in the ^{13}C NMR of commercial Fmoc-Phe-OH); HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{24}H_{22}N_2O_3Na$, 409.1528; found, 409.1535.

4-Methoxy-α-[(2-phenylacetyl)amino]benzenepropanamide (13b). The filtrate was added carefully to 25 mL of cold 2 M NaOH, and the mixture was extracted with 2 × 20 mL of DCM. The combined extracts were dried (Na_2SO_4) and concentrated to give 7.7 mg of crude material. Trituration under 2 mL of diethyl ether afforded 5.7 mg (36%) of **13b** as an amorphous solid, mp 169−70 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.75 (dd, J = 13.7 and 9.6 Hz, 1H), 2.98 (dd, J = 13.7 and 4.7 Hz, 1H), 3.28 (d, J = 14.0 Hz, 1H), 3.34 (d, J = 13.9 Hz, 1H), 3.71 (s, 3H), 4.43 (ddd, J = 9.3, 8.5, and 4.7 Hz, 1H), 6.77 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 7.05 (br s, 1H), 7.18−7.24 (m, 5H), 7.45 (br s, 1H), 8.11 (br d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 39.2, 39.6, 53.6, 54.9, 113.4, 126.0, 127.9, 128.1, 129.1, 129.8, 137.9, 157.6, 170.0, 173.0; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{18}H_{20}N_2O_3Na$, 335.1372; found, 335.1375.

α-(Acetylamino)benzenepropanamide (13c).[36] LC/MS (210 nm/ESI): 33.7 min, m/z 207 [M + H]$^+$. YMC-Pack Pro C18, 150 × 4.6 mm i.d., 3 μm, 0.75 mL/min, 10% 50:50 acetonitrile/methanol with 5 mM ammonium acetate/90% water with 5 mM ammonium acetate.

α-[(3-Methyl-1-oxobutyl)amino]benzenepropanamide (13d). The filtrate was added carefully to 25 mL of cold 2 M NaOH and the mixture was extracted with 2 × 20 mL of DCM. The combined extracts were dried (Na_2SO_4) and concentrated to give 5.7 mg of crude material. Trituration under 2 mL of diethyl ether afforded 4.0 mg (32%) of **13d** as an amorphous solid, mp 182−183 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 0.67 (d, J = 6.5 Hz, 3H), 0.75 (d, J = 6.5 Hz, 3H), 1.83 (septet, J = 6.6 Hz, 1H), 1.90 (d, J = 6.3 Hz, 2H), 2.72 (dd, J = 13.7 and 10.1 Hz, 1H), 2.99 (dd, J = 13.7 and 4.5 Hz, 1H), 4.46 (ddd, J = 10.1, 8.7, and 4.5 Hz, 1H), 7.01 (br s, 1H), 7.15−7.18 (m, 1H), 7.22−7.24 (m, 4H), 7.38 (br s, 1H), 7.91 (br d, J = 8.6 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 22.0, 22.1, 25.3, 37.5, 44.4, 53.5, 126.0, 127.8, 129.0, 138.1, 171.2, 173.3; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{14}H_{20}N_2O_2Na$, 271.1422; found, 271.1427.

α-[(Cyclopropylcarbonyl)amino)]benzenepropanamide (13e). The filtrate was added carefully to 25 mL of cold 2 M NaOH, and the mixture was extracted with 2 × 20 mL of DCM. The combined extracts were dried (Na_2SO_4) and concentrated to give 6.6 mg of crude material. Trituration under 2 mL of diethyl ether gave 4.1 mg (35%) of **13e** as an amorphous solid, mp 200−202 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 0.53−0.60 (m, 4H), 1.60−1.63 (m, 1H), 2.76 (dd, J = 13.7 and 9.5 Hz, 1H), 2.96 (dd, J = 13.7 and 5.0 Hz, 1H), 4.45 (ddd, J = 9.2, 8.5, and 5.0 Hz, 1H), 7.00 (br s, 1H), 7.17−7.20 (m, 1H), 7.23−7.28 (m, 4H), 7.42 (br s, 1H), 8.22 (br d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 6.1, 6.2, 13.3, 37.7, 53.7, 126.0, 127.9, 129.0, 138.0, 172.3, 173.1; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{13}H_{16}N_2O_2Na$, 255.1109; found, 255.1102.

***N*-[2-Amino-2-oxo-1-(phenylmethyl)ethyl]-2-naphthalenecarboxamide (13f).**[37] The filtrate was added carefully to 25 mL of cold 2 M NaOH and the mixture was extracted with 2 × 20 mL of DCM. The combined extracts were dried (Na_2SO_4) and concentrated to give 7.3 mg of crude material. Trituration under 2 mL of diethyl ether gave 5.2 mg (33%) of **13f** as an amorphous solid, mp 203−205 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 3.03 (dd, J = 13.7 and 10.7 Hz, 1H), 3.15 (dd, J = 13.7 and 4.1 Hz, 1H), 4.72 (ddd, J = 10.7, 8.6, and 4.3 Hz, 1H), 7.14−7.15 (m, 2H), 7.26 (t, J = 7.6 Hz, 2H), 7.37 (d, J = 7.2 Hz, 2H), 7.58−7.62 (m, 3H), 7.88 (dd, J = 8.6 and 1.7 Hz, 1H), 7.96 (d, J = 8.3 Hz, 2H), 8.01 (d, J = 7.6 Hz, 1H), 8.42 (br s, 1H), 8.66 (br d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77, DMSO-d_6): δ 37.2, 54.7, 124.2, 126.1, 126.6, 127.48, 127.55, 127.6, 127.9, 128.7, 129.0, 131.3, 131.9, 134.0, 138.5, 166.0, 173.2 (18 ^{13}C signals are predicted, but only 17 were observed); HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{19}N_2O_2$, 319.1441; found, 319.1447.

α-[(2*E*)-3-((4-Methoxyphenyl)-1-oxo-2-propenyl)amino]-benzenepropanamide (13g). The filtrate was immediately evaporated to dryness with a stream of nitrogen gas to give 10.5 mg of crude material that was purified by reverse-phase HPLC on a Dynamax Microsorb C-18, 5 μm column (21.4 × 250 mm) eluting isochratically with 60:40 1:1 MeCN/MeOH (with 5 mM ammonium acetate)/water (with 5 mM ammonium acetate) at 5 mL/min and detection at 254 nm to afford 3.5 mg (21%) of **13g** (95:5 mixture of E/Z isomers) as an amorphous solid, mp 191−201 °C (dec); ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 3.10 (dd, J = 13.8 and 8.0 Hz, 1H), 3.22 (dd, J = 13.8 and 5.9 Hz, 1H), 3.83 (s, 3H), 4.82 (dd, J = 13.8 and 7.3 Hz, 1H), 5.33 (br s, 1H), 5.77 (br s, 1H), 6.23 (br s, 1H), 6.26 (d, J = 15.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 7.26−7.34 (m, 5H), 7.44 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 15.5 Hz, 1H); ^{1}H NMR (500.13 MHz, CD_3OD): δ 2.99 (dd, J = 13.9 and 8.9 Hz, 1H), 3.22 (dd, J = 13.9 and 5.8 Hz, 1H), 3.84 (s, 3H), 4.77 (dd, J = 8.9 and 5.8 Hz, 1H), 6.52 (d, J = 15.8 Hz, 1H), 6.95 (d, J = 8.7 Hz, 2H), 7.22−7.24 (m, 1H), 7.30−7.31 (m, 4H), 7.46 (d, J = 15.7 Hz, 1H), 7.51 (d, J = 8.7 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 39.2, 55.87, 55.94, 115.4, 118.8, 127.8, 128.8, 129.5, 130.3, 130.6, 138.6, 142.1, 162.8, 168.8, 176.3; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{19}H_{21}N_2O_3$, 325.1547; found, 325.1552.

α-[(2,2-Dimethyl-1-oxopropyl)amino]benzenepropanamide (13h).[38] The filtrate was immediately evaporated to dryness with a stream of nitrogen gas to give crude material that was purified by reverse-phase HPLC on a Dynamax Microsorb C-18, 5 μm column (21.4 × 250 mm) eluting isochratically with 60:40 1:1 MeCN/MeOH (with 5 mM ammonium acetate)/water (with 5 mM ammonium acetate) at 5 mL/min and detection at 254 nm to afford 8.0 mg (43%) of **13h** as an amorphous solid, mp 94−96 °C; ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.12 (s, 9H), 3.09 (m, 2H), 4.69 (dd, J = 14.4 and 7.2 Hz, 1H), 5.59 (br s, 1H), 6.12 (br s, 1H), 6.30 (br d, J = 7.2 Hz, 1H), 7.22−7.25 (m, 3H), 7.28−7.31 (m, 2H); ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 27.3, 38.0, 38.7, 53.8, 127.1, 128.7, 129.3, 136.6, 173.4, 178.7; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{14}H_{21}N_2O_2$, 249.1598; found, 249.1597.

α-[(Tricyclo[3.3.1.1^{3,7}]decyl)amino]benzenepropanamide (13i). The filtrate was immediately evaporated to dryness with a stream of nitrogen gas to give crude material that was purified by reverse-phase HPLC on a Dynamax Microsorb C-18, 5 μm column (21.4 × 250 mm) eluting isochratically with 75:25 1:1 MeCN/MeOH (with 5 mM ammonium acetate)/water (with 5 mM ammonium acetate) at 5 mL/min and detection at 254 nm to afford 8.1 mg (33%) of **13i** as a glass: ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 1.64−1.67 (m, 3H), 1.71−1.74 (m, 3H), 1.76 (m, 6H), 2.01 (br s, 3H), 3.08 (m, 2H), 4.69 (dd, J = 14.2 and 6.9 Hz, 1H), 5.58 (br s, 1H), 6.06 (br s, 1H), 6.24 (br d, J = 7.1 Hz, 1H), 7.22−7.25 (m, 3H), 7.28−7.31 (m, 2H); ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 28.0, 36.4, 38.0, 39.0, 40.6, 53.6, 127.1, 128.7, 129.3, 136.6, 173.5, 178.2; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{20}H_{27}N_2O_2$, 327.2067; found 327.2064.

α-[(4-Methoxybenzoyl)amino]benzenepropanamide (13j) and *N*-(4-Methoxybenzoyl)phenylalanine (14j). In a 25 mL solid-phase peptide synthesis (SPPS) vessel, Fmoc-Phe-NH-Rink Amide MBHA resin (576 mg, 190 μmol, 0.33 mmol/g, Peptides International) was deprotected using 30% piperidine in NMP.[1] The amine resin was then treated with 3.77 mL of a 0.25 M solution of 4-methoxybenzoic acid (143 mg, 942 μmol, 4.96 equiv) in 0.25 M *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) (361 mg, 952 μmol, 5.01 equiv) in NMP followed by 3.77 mL of a 0.50 M solution of diisopropylethylamine

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(1880 μmol, 9.91 equiv) in NMP. The vessel was rocked at room temperature on an orbital shaker for 22 h and was then drained. The resin gave a negative Kaiser test[39] and was then washed with 4 × 10 mL × 2 min each successively of NMP, THF, and DCM. Acylated resin **12j** was treated with 6 mL of 95:5 (v/v) TFA/H_2O, and the vessel was rocked for 30 min at room temperature. The filtrate was collected, and the resin was washed once with 6 mL of the cleavage cocktail and once with 6 mL of DCM. The filtrate was allowed to stand at room temperature for 5 h to allow for hydrolysis to proceed partially and was then evaporated under a stream of nitrogen gas over a 3 h period (effluent gases were scrubbed with a solution of sodium hydroxide) to give 64 mg of a sticky white solid that was passed through a 500 mg cartridge of cyanosilica gel using 7:3 hexanes/acetone to elute the amide/acid mixture. After solvent removal by evaporation, the mixture was dissolved in DCM and was washed with 2 × 10 mL of 1.0 N NaOH and once with 10 mL of water and was then dried (Na_2SO_4). Concentration gave 19.6 mg (35%) of **13j** as an amorphous solid, mp 201−204 °C; ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.97 (dd, J = 13.6 and 10.7 Hz, 1H), 3.10 (dd, J = 13.6 and 4.0 Hz, 1H), 3.79 (s, 3H), 4.61 (ddd, J = 10.8, 8.6, and 4.2 Hz, 1H), 6.97 (d, J = 8.9 Hz, 2H), 7.08 (br s, 1H), 7.15 (t, J = 7.4 Hz, 1H), 7.24 (t, J = 7.5 Hz, 2H), 7.33 (d, J = 7.1 Hz, 2H), 7.51 (br s, 1H), 7.78 (d, J = 8.9 Hz, 2H), 8.32 (d, J = 8.5 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 37.1, 54.6, 55.2, 113.2, 126.0, 126.3, 127.9, 129.0, 129.1, 138.5, 161.5, 165.5, 173.4; HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{19}N_2O_3$, 299.1390; found, 299.1392.

The combined basic extracts were adjusted to pH 1 with 3 N HCl and were extracted with 2 × 25 mL of DCM. The combined extracts were dried (Na_2SO_4) and concentrated to give 12.1 mg (21%) of **14j** as an amorphous solid, mp 164−167 °C; ^{1}H NMR (500.13 MHz, CD_3OD): δ 3.11 (dd, J = 13.9 and 9.4 Hz, 1H), 3.32 (dd, J = 5.0 Hz, 1H), 3.83 (s, 3H), 4.83 (dd, J = 9.4 and 4.9 Hz, 1H), 6.94 (d, J = 8.9 Hz, 2H), 7.19 (m, 1H), 7.24−7.28 (m, 4H), 7.70 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 38.2, 55.6, 55.9, 114.7, 127.4, 127.8, 129.4, 130.3, 138.8, 164.1, 169.7, 175.0 (only 12 of 13 lines were observed); HRMS (ESI-TOF) m/z: [M + H]$^+$ calcd for $C_{17}H_{18}NO_4$, 300.1230; found, 300.1229.

Preparation of Resin 12k for Cleavage Method Study (Table 4). A 25 mL SPPS vessel was charged with 1.857 g (0.5478 mmol, 0.295 mmol/g) of Fmoc-Phe-NH-Rink Amide MBHA resin. The resin was swelled by washing with 3 × 10 mL of NMP followed by 10 mL of 20% (v/v) piperidine in NMP. The resin was then treated with 12 mL of 20% piperidine in NMP. The vessel was rocked on an orbital shaker for 40 min and was then drained, and the resin was washed with 5 × 10 mL of NMP. To the resin was then added a solution of 0.374 g (2.75 mmol, 5.02 equiv) of 4-methylbenzoic acid and 0.418 g (2.73 mmol, 4.98 equiv) of 1-hydroxybenzotriazole in 10.9 mL of NMP followed by a solution of 0.346 g (2.74 mmol, 5.00 equiv) of diisopropylcarbodiimide in 1 mL of NMP. The vessel was rocked on an orbital shaker for 6 days and was then drained, and the resin was washed with 3 × 10 mL each of NMP and THF and then with 5 × 10 mL of DCM to give **12k**, which was prepared as an isopycnic mixture using 2:1 NMP/DCM and was equally distributed to eleven 3.5 mL reaction vessels to provide 50 μmol per vessel (see General Procedure for the Preparation and Distribution of Isopycnic Suspensions of Resins). Each resin was then washed with 5 × 2 mL of DCM.

α-[(4-Methylbenzoyl)amino]benzenepropanamide (13k).[40] Compound **13k** was synthesized in solution phase using the method of Uchida et al.[41] and was purified for use as the authentic standard in the quantitative LC measurements (optimization of cleavage conditions, Table 4). A 25 mL, three-necked, round-bottomed flask fitted with a stirrer bar, thermometer, and rubber septum was charged with 0.105 g (1.25 mmol) of sodium bicarbonate, 2.0 mL of water, and 2.0 mL of acetone. The contents were cooled with an ice bath, and 0.200 g (1.22 mmol) of L-phenylalanineamide (Chem-Impex, Int.) was added in one portion. 4-Methylbenzoyl chloride (0.208 g, 1.34 mmol, 1.10 equiv), dissolved in 0.5 mL of acetone, was added dropwise via syringe. The contents were allowed to gradually warm to room temperature and were stirred for 18 h. The contents were cooled with an ice bath, and the solid was collected by suction filtration washing with a few milliliters of cold 1:1 acetone/water to afford 97 mg of crude **13k**, which was triturated under hot methanol to give 29.5 mg (9%) of the QLC calibration sample. ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 2.33 (s, 3H), 2.98 (dd, J = 13.6 and 10.7 Hz, 1H), 3.10 (dd, J = 13.6 and 4.0 Hz, 1H), 4.62 (ddd, J = 10.7, 8.6, and 4.2 Hz, 1H), 7.09 (br s, 1H), 7.15 (t, J = 7.3 Hz, 1H), 7.24 (m, 4H), 7.32 (d, J = 7.5 Hz, 2H), 7.52 (br s, 1H), 7.70 (d, J = 8.2 Hz, 2H), 8.37 (d, J = 8.4 Hz, 1H); ^{13}C NMR (125.77 MHz, DMSO-d_6): δ 20.8. 37.1, 54.6, 126.0, 127.3, 127.9, 128.6, 129.0, 131.2, 138.5, 141.0, 165.9, 173.3. LC/MS (ESI): 8.19 min, m/z 283 [M + H]$^+$.

***N*-(4-Methoxybenzoyl)-Phe-Ala-NH-Rink Amide MBHA Resin (15) and *N*-(4-Methoxybenzoyl)-Phe-Ala-O Wang Resin (16, Scheme 5).** Two 50 mL SPPS vessels were charged, respectively, with 566 mg (300 μmol) of Fmoc-Ala-O Wang resin (0.53 mmol/g, Peptides International) and 698 mg (300 μmol) of Fmoc-Ala-NH-Rink MBHA resin (0.43 mmol/g, Peptides International). To each vessel was added 20 mL of 20% (v/v) piperidine in NMP. The vessels were rocked at room temperature for 45 min and were then drained. Each resin was washed with 4 × 10 mL of NMP and was treated with 3.0 mL (750 mmol, 2.5 equiv) of 0.25 M HBTU in NMP, 3.0 mL (750 mmol, 2.5 equiv) of 0.25 M Fmoc-Phe-OH in NMP, and 3.0 mL (1500 μmol, 5.0 equiv) of 0.50 M diisopropylethylamine in NMP. The vessels were rocked overnight at room temperature. To each vessel was again added 3.0 mL (750 mmol, 2.5 equiv) of 0.25 M HBTU in NMP, 3.0 mL (750 mmol, 2.5 equiv) of 0.25 M Fmoc-Phe-OH in NMP, and 3.0 mL (1500 μmol, 5.0 equiv) of 0.50 M diisopropylethylamine in NMP. The vessels were rocked overnight at room temperature. After 48 h, Kaiser[39] test on both resins was negative. Each resin was then washed with 4 × 10 mL of NMP followed by 4 × 10 mL of DCM and then 3 × 10 mL of NMP. Piperidine (20% in NMP, 20 mL) was added to each resin, and the vessels were rocked for 45 min. The resins were washed with 4 × 10 mL of NMP and were then treated with 6.0 mL of 0.25 M (1500 μmol, 5.0 equiv) HBTU in NMP, 6.0 mL of 0.25 M (1500 μmol, 5.0 equiv) 4-methoxybenzoic acid in NMP, and 6.0 mL (3000 μmol, 10.0 equiv) of 0.50 M diisopropylethylamine in NMP. The vessels were rocked overnight at room temperature. After 24 h, each resin gave a negative Kaiser[39] test. The vessels were drained, and the resins were washed with 3 × 10 mL of NMP, 3 × 10 mL of THF, and 3 × 10 mL of DCM and then capped for storage for 3 days.

Cleavage/Hydrolysis of Resins 15 and 16 to 17 and 18 (Scheme 5). Isopycnic suspensions of Rink resin **15** (300 μmol) and Wang resin **16** (300 μmol) were each equally distributed, respectively, to six 3.5 mL reaction vessels in rows A and B of two 2 × 3 Bill-Boards (50 μmol of resin per vessel). All vessels of one Bill-Board were then treated with 1.5 mL of 95:5 TFA/water and were rotated for 30 min at room temperature. The vessels were drained into 4-dram, tooled-neck collection vials, and each resin was rinsed with 2 mL of 95:5 TFA/water. Filtrates from A1 and B1 were sampled and evaporated to dryness and analyzed (0.5 h time point), filtrates from A2 and B2 were allowed to stand at room temperature for 6 h and were then analyzed by LC/MS, and then the filtrates from A3 and B3, which had stood for 24 h, were analyzed. Each vial assayed at 30 min was immediately stored at −20 °C overnight and was concentrated under a stream of nitrogen gas to afford 10.9 mg of crude **17** and 15.2 mg of crude **18**.

***N*-(4-Methoxybenzoyl)-L-phenylalanyl-L-alaninamide (17).** The crude material (9.3 mg) was purified by cyanosilica gel chromatography (500 mg prepacked column, eluting with 1:1 and then 4:6 hexanes/acetone) followed by reverse-phase HPLC on a Dynamax Microsorb C-18, 5 μm column (21.4 × 250 mm) using the isochratic system of 55:45 1:1 MeCN/MeOH (5 mM ammonium acetate)/water (5 mM ammonium acetate) at a 5 mL/min flow rate to give 2.1 mg (11%) of **17** as an amorphous solid, mp 229−231 °C; ^{1}H NMR (500.13 MHz, CD_3OD): δ 1.36 (d, J = 7.2 Hz, 3H), 3.06 (dd, J = 13.8 and 9.2 Hz, 1H), 3.26 (dd, J = 13.9 and 6.0 Hz, 1H), 3.84 (s, 3H), 4.34 (q, J = 7.2 Hz, 1H), 4.79 (dd, J = 9.1 and 5.9 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 7.20 (m, 1H), 7.26−7.32 (m, 4H), 7.72 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 18.2, 38.6, 50.1, 55.9, 56.7, 114.7, 127.2, 127.9, 129.5, 130.37, 130.39, 138.6, 164.1, 169.9,

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173.6, 177.3; HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{24}N_3O_4$, 370.1761; found, 370.1767.

***N*-(4-Methoxybenzoyl)-L-phenylalanyl-L-alanine (18).** The crude material (12.9 mg) was chromatographed using a 500 mg prepacked cyanosilica cartridge (Thermo Scientific) and eluted with hexanes/acetone (7:3 and 6:4) to afford 4.8 mg (26%) of **18** as an amorphous solid, mp 181−184 °C (dec); ^{1}H NMR (500.13 MHz, CD_3OD): δ 1.42 (d, J = 7.3 Hz, 3H), 3.03 (dd, J = 14.0 and 9.7 Hz, 1H), 3.29 (dd, J = 14.4 and 4.9 Hz, 1H), 3.83 (s, 3H), 4.39 (q, J = 7.2 Hz, 1H), 4.86 (dd, J = 9.8 and 4.9 Hz, 1H), 6.94 (d, J = 8.9 Hz, 2H), 7.18 (t, J = 7.3 Hz, 1H), 7.25 (t, J = 7.5 Hz, 2H), 7.31 (d, J = 7.1 Hz, 2H), 7.69 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125.77 MHz, CD_3OD): δ 18.0, 38.9, 49.9, 55.9, 56.3, 114.7, 127.3, 127.7, 129.4, 130.3, 130.4, 138.7, 164.1, 169.7, 173.5, 176.3; HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{20}H_{23}N_2O_5$ 371.1601; found 371.1604.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR data and spectra and graphical treatments of the Hammett and cleavage method studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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