Model Studies for New *o*-Nitrobenzyl Photolabile Linkers: Substituent Effects on the Rates of Photochemical Cleavage

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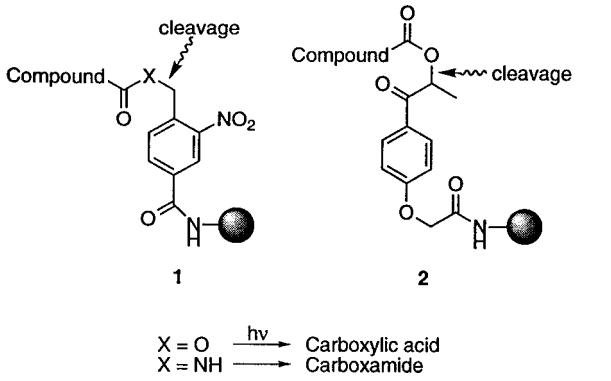
Both a model phenacyl and *o*-nitrobenzyl photolabile linker from the literature along with four new *o*-nitrobenzyl linkers were prepared and the kinetics of their photolytic cleavage examined in solution. The linkers were prepared by amidation of the carboxylic acid anchoring tether with benzylamine, and the cleavable benzylic substituent was chosen to be either acetic acid or acetamide. Irradiation of the linkers in four solvents (methanol, *p*-dioxane, and aqueous buffer ± dithiothreitol) at 365 nm and analysis via HPLC afforded kinetic rates of cleavage suitable for comparative purposes. The phenacyl linker was found to cleave slowly under aqueous conditions with no detectable cleavage being observed in the organic solvents. Known *o*-nitrobenzyl linker **4** showed modest rates of cleavage in aqueous and organic solvents. Incorporation of two alkoxy groups in the benzene ring to generate the veratryl-based linker **13a** increased the rate of cleavage dramatically, and introduction of an additional benzylic methyl group (**13b**) increased the rate of cleavage by an additional 5 fold. Increasing the length of the anchoring carboxylic acid tether from acetic to butyric acid (**19**) improved the cleavage kinetics modestly in organic media and slightly diminished the rates in water. The amide model linker **21** cleaved from 3 to 7 times faster than the corresponding ester linkage **19**. An amide-generating linker **26** was prepared, and its performance to generate photolabile solid supports was briefly examined. The stability of the linker and subsequent cleavage upon photolysis from the support of an isotopically enriched 4-thiazolidinone was demonstrated by gel phase ^{13}C NMR.

Introduction

The rapidly growing field of combinatorial chemistry involving libraries of molecules has renewed interest in the use of solid phase organic synthesis techniques as a convenient means of assembling molecules.[1,2] As the need for diversity of chemistry amenable to a solid phase approach has greatly expanded, so too has the need for improved and novel linkers anchoring the molecules to the support. While many groups have focused their efforts on chemically-cleavable linkers (i.e. acid-, base-, transition metal-cleavable linkers), we have been pursuing linkers which can be cleaved with UV light. Photolytic cleavage offers a mild method of cleavage from the support which is particularly attractive in combinatorial library screening, as the release of the molecules can occur after removal of any protecting groups and extensive washing of the resin, thereby affording the liberated molecules suitable for biological assay without contamination by cleavage reagents. Photolabile linkers also afford additional flexibility in compound synthesis as many linkers are stable to both acidic and basic conditions suitable for small molecule synthesis. The use of a photolabile molecule[3] as an orthogonal linker for the cleavage of molecules from solid supports dates back two decades since the *o*-nitro benzyl support **1** derived from 4-(bromomethyl)-3-nitrobenzoic acid was first described by Rich.[4] Support **1** has been widely employed by many researchers for the generation of both peptide acids and amides[5] and recently small molecules[6] and tagging moieties.[7] Several phenacyl linkers first described by Wang[8] and later evolved to the *p*-alkoxy derivative **2**[9] have also been attached to supports to afford peptide acids, although less extensively than the *o*-nitrobenzyl supports.



[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1997.

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(2) Solid phase organic synthesis reviews: (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (b) Früchtel, J. S.; Jung, G. *Angew. Chem.* **1996**, *35*, 17−41. (c) Patel, D. V.; Gordon, E. M. *Drug Disc. Today* **1996**, *4*, 134−144.

(3) For a general review of the use of photolabile supports see: Lloyd- Williams, P.; Albericio, F.; Giralt, E. *Tetrahedron* **1993**, *49*, 11065−11133. For the general use of photoprotecting groups see: Pallai, V. N. R. *Synthesis* **1980**, 1−26.

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(5) (a) Rich, D. H.; Gurwara, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 1575−1579. (b) Rich, D. H.; Gurwara, S. K. *Tetrahedron Lett.* **1975**, 301−304. (c) Hammer, R. P.; Albericio, F.; Gera, L.; Barany, G. *Int. J. Pept. Protein Res.* **1990**, *36*, 31−45. (d) LLoyd-Williams, P.; Albericio, F.; Giralt, E. Int. *J. Pept. Protein Res.* **1991**, *37*, 58−60. (e) Lloyd-Williams, P.; Gairí, M; Albericio, F.; Giralt, E *Tetrahedron* **1993**, *49*, 10069−10078. (f) Giralt, E.; Albericio, F.; Dalcol, I.; Gairí, M.; Lloyd-Williams, P.; Rabanal, F. In *Innovation and Perspectives in Solid Phase Synthesis*; Epton, R., Ed.; Mayflower Worldwide Ltd.: Birmingham, UK, 1994; pp 51−60.

(6) (a) Burbaum, J. J.; Ohlmeyer, M. H. J.; Reader, J. C.; Henderson, I.; Dillard, L. W.; Li, G.; Randle, T. L.; Sigal, N. H.; Chelsjy, D.; Baldwin, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6027−6031.

(7) Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922−10926.

(8) Wang, S. W. *J. Org. Chem.* **1976**, *41*, 3258−3261.

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Although the original photolabile supports and their close analogs have been widely employed in the peptide arena, their usefulness in small molecule synthesis remains to be validated. As the realm of solid phase chemistries is expanded beyond biopolymers, the interplay between rate of cleavage from the support and any inherent instability the molecules may have to UV light becomes increasingly important. Peptides are relatively stable to >350 nm UV light, whereas complex heterocycles may not be stable, and hence minimizing overexposure to UV light is crucial to the success of such photolinkers. Both the *o*-nitrobenzyl and phenacyl linkers suffer from unduly slow cleavage kinetics, with typical photolysis times reported in the literature ranging from 12 to 24 h.[3,5] These long photolysis times may in fact serve to exacerbate the undesired photooxidation of sensitive functionalities.[10] Methionine for example, has been observed to be particularly sensitive to photooxidation during cleavage.[5] Moreover, the *o*-nitrobenzyl linkers generate a reactive nitroso-aldehyde on the support upon cleavage which can potentially trap any liberated compound or may act as an internal light filter to slow the rate of cleavage.[11] The principal chemical liability of the phenacyl linker **2** appears to be the reactive carbonyl group of the linker participating in undesired cyclizations and small amounts of diketopiperazine formation while at the dipeptide stage.[9b,12] The sensitivity of linker **2** toward mildly nucleophilic agents[9] additionally restricts the range of chemistries possible with supports bearing linkers of this type.

Other researchers have addressed these shortcomings and several additional photocleavable linkers have appeared recently. Pillai introduced an α methyl group onto the benzylic carbon of an *o*-nitrobenzyl linker, but observed poor release of peptides longer than five residues, presumably due to poor swelling of the resin.[13] The use of a 2′-nitrobenzhydryl support has also been reported to give high yields of peptide acids[14] and of peptide amides[15] after 20−24 h of photolysis. Brown has introduced a new linker with particular application for the mass spectral characterization of compounds released from it.[16] Greenberg has described several *o*-nitrobenzyl linkers and documented their use in DNA synthesis.[17] Chan has reported a new linker based on 3′-methoxybenzoin.[18] We recently described two new *o*-nitrobenzyl linkers based on α-methyl-6-nitroveratrylamine and α-methyl-6-nitroveratryl alcohol.[19] The use of veratryl-based nitrobenzyl groups which incorporate two additional alkoxy groups onto the benzene ring greatly facilitates the photolytic cleavage with >350 nm UV light by shifting the principle chromophore into this region (*vide infra*). The inclusion of an α-methyl group onto the benzylic carbon atom enhances the relative cleavage kinetics and additionally renders the photolysis byproducts much less reactive toward the liberated compounds.[20] The rapid cleavage rates and minimally-reactive byproducts have allowed us to obtain rapid and high yields of peptides containing sensitive amino acids including methionine,[19] as well as affording high yields of small molecules such as thiazolidinones[21a] and *β*-lactams.[21b] In developing these new linkers we had occasion to examine their photokinetic properties in solution as well as the cleavage properties of several related analogs and compared them to those of known linkers **1** and **2**. We now wish to report on these findings.

Results and Discussion

The most common method of preparing photolabile supports is to anchor preformed linkers[22] bearing a carboxylic acid tether onto the support as amides, thereby affording maximum control over the chemistry and level of substitution of the support. The alternative approach of synthesizing the linker *in situ* on the support has at least in one case led to difficulties due to competing chemistries of the linker and the polymer support.[13] Amide-generating linkers are thus incorporated as protected (e.g. Boc or Fmoc) amino acids whereas acid-generating linkers are typically attached as hydroxymethyl acids, which are subsequently esterified by a number of methods.[2] We chose to initially survey the photolysis kinetics of several model linkers in *solution* to access their relative cleavage rates in order to determine what effects differing substituents about the ring would have. The best of these linkers would then be transferred to the support and their usefulness confirmed for solid phase organic synthesis. The model linkers were prepared as either their O- or N-acetates to represent a generic acid or amide release, respectively, and were amidated with benzylamine on their carboxy terminus to mimic the local environment which would be present on a typical solid support. It is difficult to extrapolate relative cleavage rates for linkers **1** and **2** from the data reported in the literature, as it is not yet common to report UV light intensity when describing photolysis conditions.[23] We therefore prepared and investigated the solution photokinetics of benzylamide **4** as an analog of **1** and prepared benzylamide **7** as an analog of **2** in order to directly compare the new linkers under investigation with those previously employed.

(9) (a) Tjoeng, F. S.; Heavner, G. A. *J. Org. Chem.* **1983**, *48*, 355−359. (b) Bellof, D.; Mutter, M. *Chimia* **1985**, *39*, 317−320.

(10) The undesired oxidation most likely stems from the prolonged exposure to UV light and not from interaction of the products with the resin-bound photolysis byproducts. Workers have noted that photolysis of methionine in solution for 16 h in the absence of an *o*-nitrobenzyl linker resulted in a 1:1 mixture of methionine:methionine sulfoxide; see ref 5c.

(11) (a) Patchnornik, A.; Amit, B.; Woodward, R. B. *J. Am. Chem. Soc.* **1970**, *92*, 6333−6335. (b) Reid, W.; Wilk, M. *Justus Liebigs Ann. Chem.* **1954**, *590*, 91.

(12) Tjoeng, F. S.; Tam, J. P.; Merrifield, R. B. *Int. J. Pept. Protein Res.* **1979**, *14*, 262−274.

(13) Ajayaghosh, A; Pillai, R. *Tetrahedron* **1988**, *44*, 6661−6666.

(14) Ajayaghosh, A.; Pillai, V. N. R. *J. Org. Chem.* **1987**, *52*, 5714−5717.

(15) Ajayaghosh, A.; Pillai, V. N. R. *Tetrahedron Lett.* **1995**, *36*, 777−780.

(16) Brown, B. B.; Wagner, D. S.; Geysen, H. M. *Mol. Diversity* **1995**, *1*, 4−12.

(17) (a) McMinn, D. L.; Greenberg, M. M. *Tetrahedron* **1996**, *52*, 3827−3840. (b) Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525−529. (c) Yoo, D. J.; Greenberg, M. M. *J. Org. Chem.* **1995**, *60*, 3358−3364. (d) Greenberg, M. M.; Gilmore, J. L. *J. Org. Chem.* **1994**, *59*, 746−753.

(18) Rock, R. S.; Chan, S. I. *J. Org. Chem.* **1996**, *61*, 1526−1529.

(19) (a) Holmes, C. P.; Jones, D. G. *J. Org. Chem.* **1995**, *60*, 2318−2319. (b) Holmes, C. P.; Jones, D. G.; Frederick, B. T.; Dong, L.-C. In *Peptides: Chemistry, Structure and Biology (Proceedings of the 14th American Peptide Symposium)*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd: Birmingham, 1995; pp 44−45.

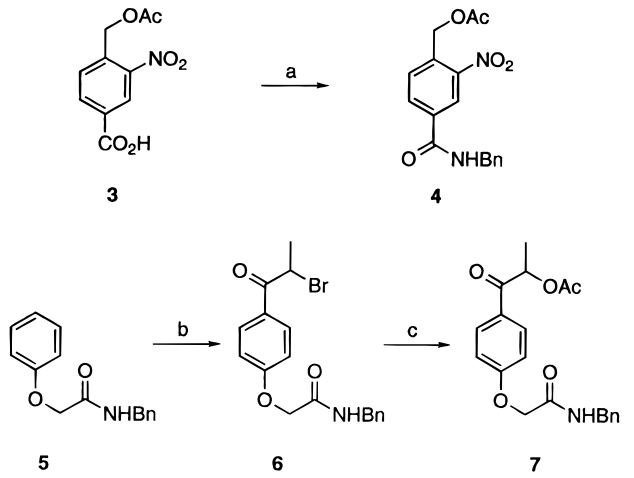
(20) The molecules lead to the nitrosoacetophenone; the characterization and subsequent reactivity of the nitrosoacetophenone photoproduct will be reported in due course.

(21) (a) Holmes, C. P.; Chinn, J. P.; Look, G. C.; Gordon, E. M.; Gallop, M. A. *J. Org. Chem.* **1995**, *60*, 7328−7333. (b) Ruhland, B.; Bhandari, A.; Gordon, E. M.; Gallop, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 253−254.

(22) Alternatively referred to as "handles".

(23) Since photolytic cleavage is first order with respect to UV light intensity, it is the combination of lamp intensity *and* photolysis time (in addition to wavelength) which should be reported to quantify the amount of light delivered.

Scheme 1[a]



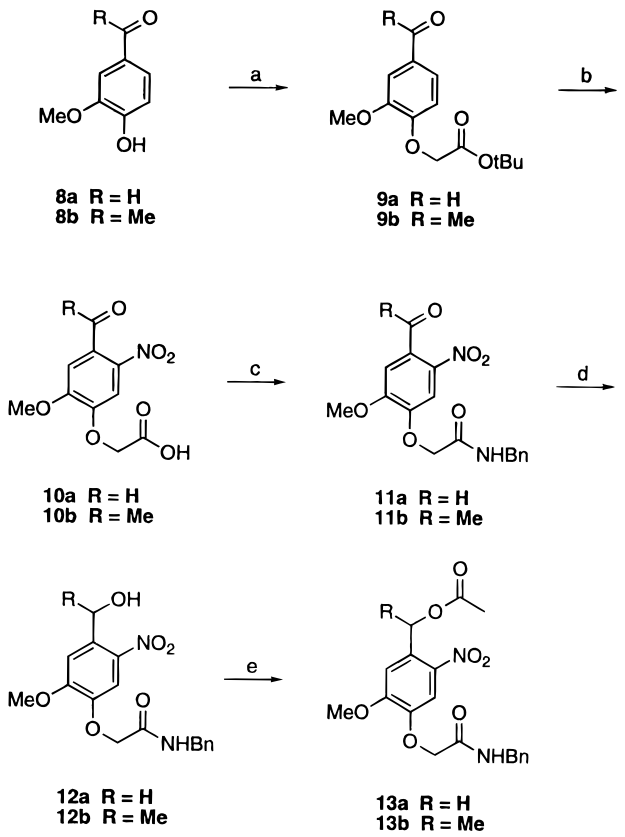
[a] Reagents: (a) benzylamine, DCC, HOBt, DMF; (b) 2-bromopropionyl chloride, $AlCl_3$, CS_2; (c) HOAc, DIEA, EtOAc.

Model linker **4** was synthesized via amidation of benzoic acid **3**[24] with benzylamine and DCC (Scheme 1). The phenacyl derivative **7** was prepared starting with amide **5**[25] via acylation with 2-bromopropionyl chloride to generate intermediate bromoacyl amide **6** in 85% yield. Partial halogen exchange was observed during the Friedel−Crafts reaction to afford **6** contaminated with approximately 5% of the corresponding chloro compound.[26] This proved to be of no concern as subsequent displacement of the halogen(s) with acetic acid gave the desired phenacyl model linker **7** in 82% yield.

Synthesis of two veratryl-based linkers started with either vanillin or acetovanillone (Scheme 2). Alkylation of vanillin **8a** with *tert*-butyl bromoacetate gave the aldehyde-ester **9a** in quantitative yield. Subsequent nitration with 70% nitric acid effected both the ring nitration and cleavage of the *tert*-butyl ester to give the acid **10a** in 91% yield. Amidation with benzylamine gave the amide **11a** in 72% yield, which was reduced with $NaBH_4$ to give the benzyl alcohol **12a** in low yield. Alcohol **12a** proved to be surprisingly insoluble in most organic solvents and was difficult to prepare in modest scale. It was found that a two-step, one-pot conversion of the aldehyde group to the acetoxymethyl group afforded the best yields of model linker **13a**, which was obtained in 70% overall yield from **11a**. Conversion of acetovanillone **8b** to model linker **13b** proceeded without incident in analogy to the above procedure. Thus, alkylation of **8b** with *tert*-butyl bromoacetate, followed by nitration, amidation with benzylamine, and conversion of the ketone to the acetoxyethyl moiety afforded ample quantities of model linker **13b** for photokinetic evaluation.

The nature of the tether anchoring the linker to the support has played an important role in the usefulness of various linkers in solid phase synthesis.[27] We were curious to explore what effect increasing the relative electron-donating ability of the para substituent may

Scheme 2[a]



[a] Reagents: (a) *tert*-butyl bromoacetate, K_2CO_3, DMF; (b) HNO_3, Ac_2O; (c) benzylamine, EDC, HOBt, DMF; (d) $NaBH_4$, THF; (e) Ac_2O, pyridine, CH_2Cl_2.

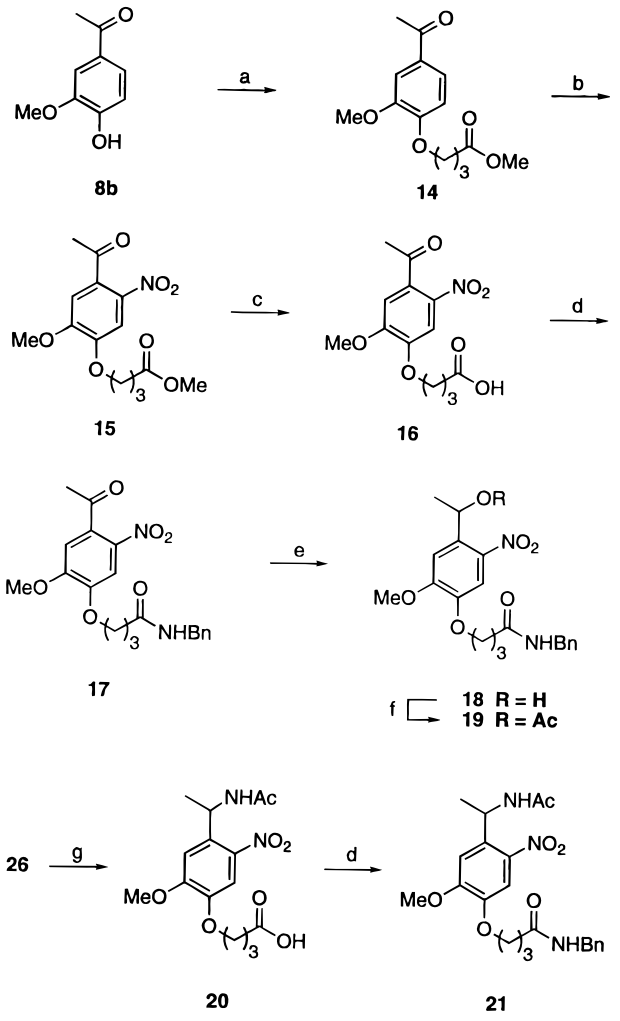
have on the photolytic cleavage. We therefore prepared linker **19** which possesses a butyric acid anchoring tail in contrast to the acetic tail present in linkers **7**, **13a**, and **13b**. Greenberg has recently described extending the tether to butyric acid after observing β-elimination with propionic acid as the tether.[17a] Linker **19** was assembled via a five-step sequence beginning with acetovanillone **8b** (Scheme 3). Alkylation of **8b** with methyl 4-bromobutyrate afforded the keto-ester **14** in quantitative yield and subsequent nitration afforded the nitrated compound **15** in 66% yield. Saponification of the ester and conversion to the corresponding benzyl amide generated amide **17** in excellent overall yield. Reduction of the ketone with $NaBH_4$ followed by acetylation afforded the desired linker **19** in near quantitative yield for the two steps.

Model amide linker **21**, which would represent a typical acylation of a support-bound amine, was additionally prepared to compare the relative rates of an amide bond cleavage. A one-pot, two-step conversion of the Fmoc-protected linker **26** (described later in this paper) to the acetate-acid **21** proceeded in 88% yield. Amidation with benzylamine gave the desired model linker **21** in good yield.

The UV spectra of the model linkers exhibited the anticipated *bathochromic* shift of the principle absorbance bands upon substitution of the phenyl ring by alkoxy groups (Figure 1). Thus, the veratryl-based linkers **13a** and **13b** showed a dramatic increase in absorbance in the targeted deprotection wavelength region centered near 365 nm in comparison to linkers **4** and **7**. Our previous work and work from others had

(24) Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936−4942.
(25) Dermer, O. C.; King, J. *J. Org. Chem.* **1943**, *8*, 168−173.
(26) Drefahl, G.; Fisher, F. *Justus Liebigs Ann. Chem.* **1956**, *598*, 159.
(27) The heightened susceptibility toward acidic cleavage as one increases the chain length of the anchoring tail is well known; see for example ref 2 and Albericio, F.; Barany, G. *Int. J. Pept. Protein Res.* **1990**, *30*, 206−216.

Scheme 3[a]



[a] Reagents: (a) methyl 4-bromobutryate, K_2CO_3, DMF; (b) HNO_3, Ac_2O; (c) NaOH, MeOH; (d) benzylamine, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride, HOBt, DMF; (e) $NaBH_4$, THF; (f) Ac_2O, pyridine, CH_2Cl_2; (g) (i) NaOH, MeOH, (ii) Ac_2O, dioxane/H_2O.

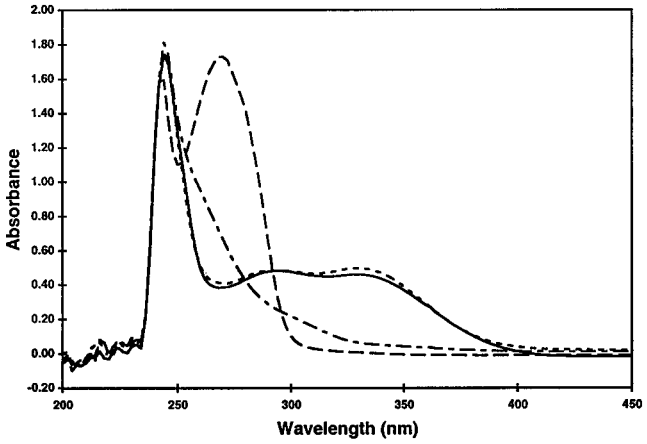


Figure 1. UV trace for photolabile linkers in *p*-dioxane (0.1 mM): (– - –) **4**; (– – –) **7**; (- - -) **13a**; (—) **13b**.

demonstrated that both peptides[28a] and oligonucleotides[28b,c] are relatively stable to photolysis with 365 nm UV light which can be easily achieved from a Hg ARC lamp or transilluminator. Linkers **19** and **21** exhibited essentially the same UV spectra as **13a** and **13b** and are not shown for clarity.

Table 1. Measured Photolysis[a] Half Lives for the Photolabile Linkers (min)

		solvent[b]		
linker	PBS	DTT/PBS	MeOH	*p*-dioxane
7	348	259	*c*	*c*
4	14.1	47.2	362	36.8
13a	12.9	7.32	16.2	4.00
13b	1.74	1.98	5.81	0.64
19	2.87	2.86	3.85	0.50
21	0.69	0.66	0.51	0.17

[a] Photolysis in indicated solvent containing 1% DMSO with Hg(Xe) Arc lamp, 350−450 nm wavelength, and a power of 10 mW/cm^2 at 365 nm. [b] PBS is phosphate-buffered saline, pH 7.4; DTT/PBS is 10 mM DTT in pH 7.4 PBS. [c] No photolysis was observed.

The photolysis rates of the model linkers were measured by photolyzing aliquots of the linkers in solution for various times and determining the rate of disappearance of the starting linker via HPLC analysis. While this technique did not allow us to quantify the amount of product produced, it did provide an expedient method to achieve our goal, namely the determination of the *relative* cleavage kinetics for the compounds. The lamp used was a 1000 W Hg(Xe) Arc lamp equipped with a 350−450 dichroic reflector thus affording primarily 365 nm illumination. The power level was adjusted to 10 mW/cm^2.[29] Times were chosen to cleave from 1 to 10% of the starting material in order to derive kinetic parameters, although the cleavage showed excellent linearity over the course of complete cleavage. No temperature control of the sample was attempted as the sample did not warm appreciably over the course of photolysis. Four solvents were chosen for the photolysis study: MeOH and *p*-dioxane to represent protic and aprotic organic solvents, respectively, whereas pH 7.4 phosphate buffer (PBS) with and without 10 mM dithiothreitol (DTT) were chosen to represent typical biological assay buffers containing an optional scavenger.[30] Table 1 summarizes the observed kinetics for the six linkers examined with the rates expressed as photolysis half-lives.

The phenacyl linker **7** was found to photolyze much more slowly in comparison to the other linkers examined (Table 1). The cleavage of **7** is strikingly solvent dependent, and we could not observe any cleavage in organic solvents under the conditions examined, whereas modest rates of cleavage was noted for the aqueous solvents. Given that previous researchers have successfully employed phenacyl linkers such as **7**, we conclude that substantially higher light intensities must have been utilized in order to achieve the reported yields. The parent *o*-nitrobenzyl linker **4** exhibited a moderate increase in photolysis kinetics and displays an interesting dichotomy: its cleavage appears abnormally fast in the aqueous environment lacking DTT relative to the organic solvents. This is in contrast to the other model *o*-nitrobenzyl linkers described in this paper and related compounds examined in our laboratory and is similar to the observed solvent dependence of phenacyl linker **7**. The presence of the additional carbonyl in conjugation

(28) (a) Holmes, C. P.; Adams, C, L.; Kochersperger, L. M.; Mortensen, R. B.; Aldwin, L. A. *Biopolymers* **1995**, *37*, 199−211. (b) Pease, A. C.; Solas, D.; Sullivan, E. J.; Cronan, M. T.; Holmes, C. P.; Fodor, S. P. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5022−5026. (c) Pirrung, M. C.; Bradley, J.-C. *J. Org. Chem.* **1995**, *60*, 116−117.

(29) For comparison, a typical hand-held UV light source emits 2−3 mW/cm^2.

(30) Walker, J. W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, *110*, 7170−7177.

with the principle nitrobenzyl chromophore may play a role in distinguishing **4** from the other *o*-nitrobenzyl linkers.

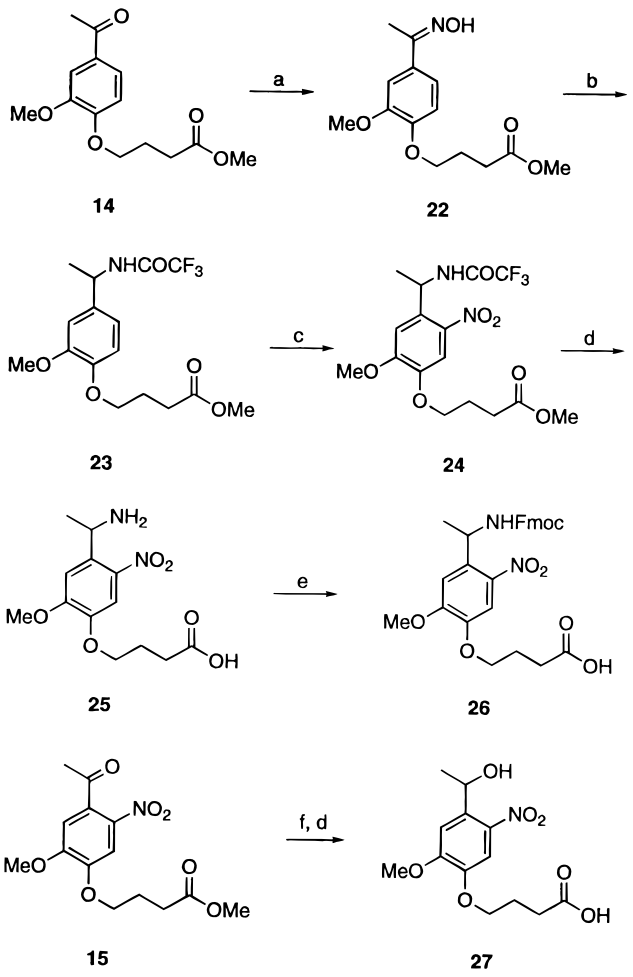
Gratifyingly, the veratryl-based linkers **13a**, **13b**, **19**, and **21** exhibited the anticipated increase in photolysis rates upon 365 nm irradiation relative to the parent *o*-nitrobenzyl linker **4**, presumably due to the increase in absorbance in this region. The kinetics of cleavage of **13a** are representative of this class and similar to what we have observed for related *o*-nitrobenzyl protecting groups, namely the decrease in rate under protic conditions relative to aprotic environments. Thus, the photolysis rates of **13a** displays a three-fold rate increase when changing from PBS and to dioxane as solvent. The effect of the α-methyl group is apparent when comparing **13b** to **13a**, which shows roughly a five-fold increase in relative cleavage rates when the α-methyl substituent of **13b** is present. Hess and co-workers have also observed a five-fold increase in the rate of decay of the key *aci*-nitro intermediate when comparing an α-proton versus α-methyl as benzylic substituents in a related series of *o*-nitrobenzyl protecting groups.[31]

Model linker **19** with the longer anchoring tail exhibited faster kinetics in the organic solvents compared to the shorter tail analog **13b**, yet curiously this trend reversed itself under the aqueous conditions. The dramatic increase in photolysis rates observed in organic solvents for linkers **13b** and **19**, which is 60−90 times faster than the previously known linker **4** under identical conditions, serves to illustrate the kinetic advantage of these new linkers. The rapid photolysis (2−3 min half lives) for **13b** and **19** in an aqueous environment also bodes well for the use of linkers such as these for the photolytic release of compounds under biologically-relevant conditions. The linkers containing an α-methyl group do not display any dramatic kinetic dependence on whether DTT is present, whereas linkers **4** and **13a** lacking a benzylic substituent show a kinetic effect. While the mode of action of DTT is thought to intercept a key intermediate with *o*-nitrobenzyl molecules,[30] it is less clear how DTT could account for the modest increase in photolysis rate of the phenacyl linker **7**.

The amide-releasing linker **21** displayed the fastest kinetics of all the model linkers examined. Its kinetic profile is in agreement with the other α-methyl veratryl-based linkers, namely faster photolysis times being observed in organic environments and being relatively insensitive to the presence of DTT. The 10 *second* half life for cleavage of **21** observed in dioxane as compared to 36 *minutes* for **4** underscores the advantages that the veratryl-based linkers impart.

Having identified the benefit of the butyric acid tether and the α-methyl group in the model series, the next step was to assemble linkers suitable for attaching to solid supports and to explore their use. We chose to initially explore the use of an amide-generating linker and embarked on the synthesis of the fluorenylmethoxycarbonyl (Fmoc) protected amine linker **26** (Scheme 4). A route to **26** was initially explored involving the reductive amination of ketones **15** or **16**, but this proved to be intractable as no satisfactory conditions could be found.[32]

Scheme 4[a]



[a] Reagents: (a) $H_2NOH{\cdot}HCl$, pyridine/H_2O; (b) (i) H_2, Pd/C, HOAc, (ii) TFAA, pyridine; (c) HNO_3; (d) NaOH, MeOH; (e) Fmoc-Cl, H_2O/dioxane; (f) $NaBH_4$, THF.

The somewhat longer approach involving a two-step transformation of the ketone to an amino group was therefore optimized to afford sufficient quantities of **26** for further study. Treatment of **14** with hydroxylamine occurred in quantitative yield to afford crystalline oxime **22**, which could be used without any purification. A two-step procedure of hydrogenation to give an intermediate amine, followed by acylation with TFAA in pyridine gave the trifluoroacetate **23** in 80% overall yield from acetovanillone **8b**. We found pyridine to be crucial to obtain high yields during the acylation with TFAA, as the liberated TFA if not neutralized decomposed the intermediate amine, which itself bears similarity to conventional acid-cleavable linkers. Trifluoroacetate was chosen to render **23** stable to the strongly acidic conditions of nitration (70% HNO_3), which was found to give **24** in 81% yield after recrystallization. Once the nitro group is installed in the benzene ring the molecule was quite stable to acidic conditions (*vide infra*). Removal of both the trifluoroacetyl and methyl ester protecting groups was accomplished upon treatment with NaOH in refluxing MeOH/H_2O to generate the free amino acid **25**, which could be protected in the same flask by simply concentrating the reaction mixture and treating with 9-fluorenylmethyl chloroformate. In this manner we could obtained a yield of 81% for the desired photolabile linker **26**. Of particular note is that this seven-step procedure from commercially available acetovanillone does not

(31) Ramesh, D.; Wieboldt, R.; Billington, A. P.; Carpenter, B. K.; Hess, G. P. *J. Org. Chem.* **1993**, *58*, 4599−5605.

(32) Other workers have reported poor yields for the reductive amination of 2-nitro-4,5-dimethoxyacetophenone: Wilcox, M.; Viola, R. W.; Johnson, K. W.; Billington, A. P.; Carpenter, B. K.; McCray, J. A.; Guzikowski, A. P.; Hess, G. P. *J. Org Chem.* **1990**, *55*, 1585−1589.

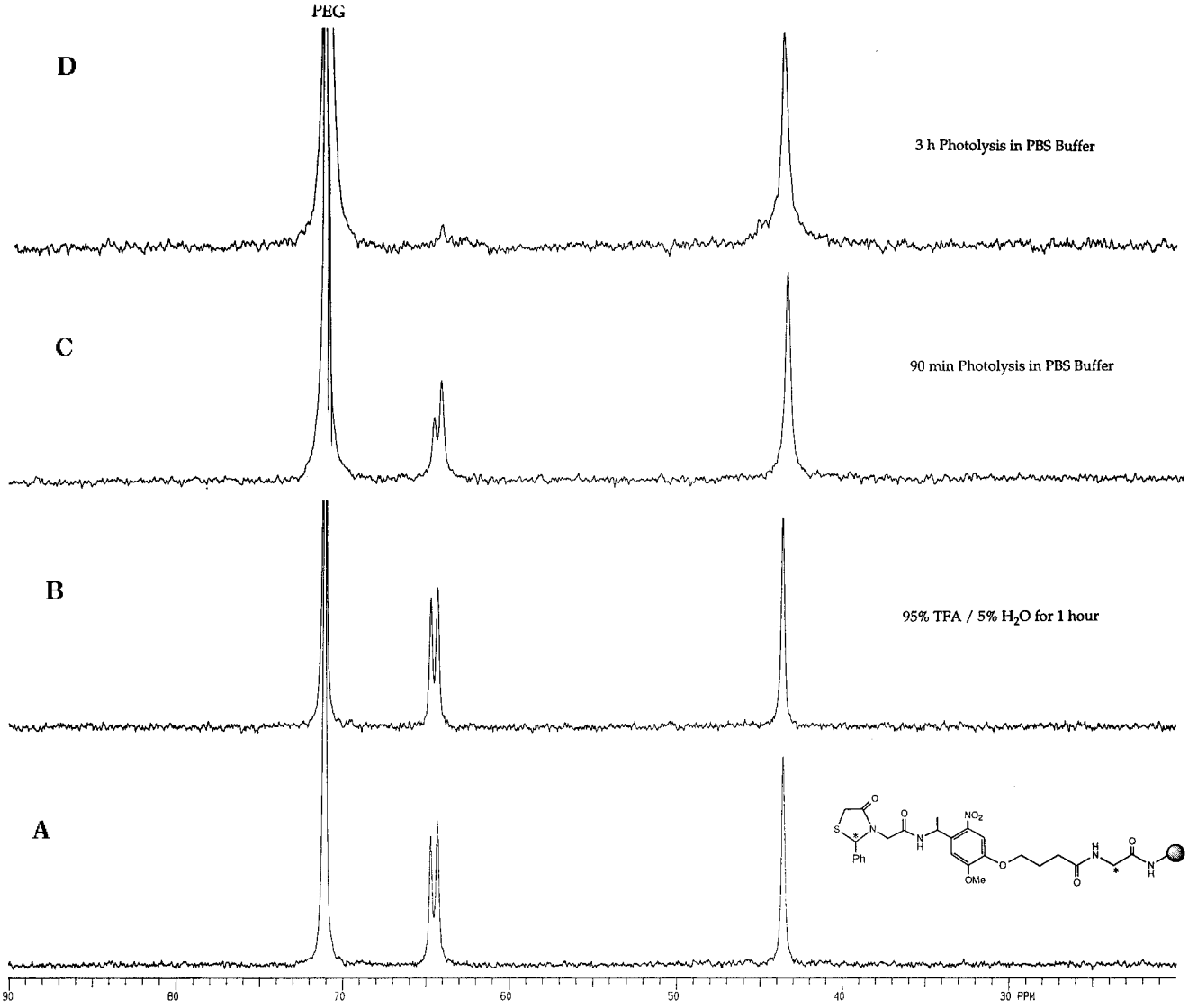


Figure 2. Gel phase ^{13}C NMR trace for resin **28**. Trace A: natural abundance polyethylene glycol (PEG) is at 71 ppm, ^{13}C-C_2 glycine appears at 44 ppm, and ^{13}C-C_2 of the thiazolidinone appears at 65 ppm. Trace B: resin **28** after exposure to TFA/H_2O) for 1 h. Trace C: resin **28** after irradiation at 365 nm for 1 h. Trace D: resin **28** after irradiation at 365 nm for 3 h.

require any chromatography, as all the intermediates are solids which were purified by recrystallization. The alcohol-based linker **27** was prepared for solid phase studies through a two-step, one-pot conversion of keto ester **15** to the intermediate hydroxy ester, followed by saponification to **27** (Scheme 4). The synthetic routes to both **26** and **27** are easily scaleable, and we have been able to prepare both on a 0.5 kg scale without incident.[33]

The chemistry of linker **26** was first explored as an amide-generating linker for both peptides[19a] and small molecules.[21] Coupling of the acid anchoring tail as an amide to a variety of commercial amine-supports with standard carbodiimide coupling agents affords quantitative loadings to generate the corresponding photolabile supports. Despite the support's sensitivity toward photolytic cleavage, the use of subdued laboratory lights or foil-wrapped vessels has generally allowed us to employ these supports without altering routine operations.

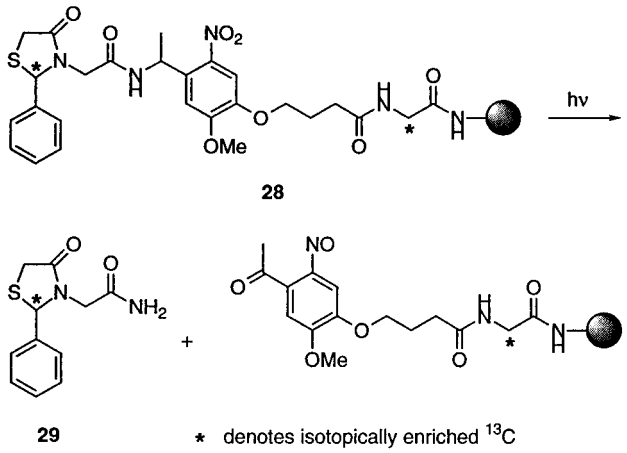
Linker **26** was used to illustrate the synthesis and cleavage of a typical support-bound small molecule, namely 4-thiazolidinone **29** (Figure 2). In addition to analyzing the supernatant from irradiated supports via HPLC to assess the quality of the liberated material, we sought to employ direct analysis of the support via fast ^{13}C NMR[34] to establish complete cleavage of the bound compound(s). We therefore assembled support **28** containing linker **26** coupled to C-α ^{13}C labeled glycine as internal reference and subsequently formed a thiazolidinone derived from a second molecule of glycine, ^{13}C labeled benzaldehyde. and mercaptoacetic acid using conditions previously described.[21a] The use of isotopically enriched reagents meant that analysis of the resin could be performed rapidly in a conventional NMR probe, thereby affording a simple tool to provide qualitative information on the performance of the linker(s). Analysis of support **28** via gel phase ^{13}C NMR showed revealed two resonances for the diastereomeric C-2 of the thiazolidinone and a single resonance for the glycine carbon (Figure 2a). The polyethylene glycol tether of the support[35] appears as a sharp singlet at ≈71 ppm. The

(33) Baer, T. A.; Raillard, S. P. Unpublished results.

(34) Look, G. C.; Holmes, C. P.; Chinn, J. P.; Gallop, M. A. *J. Org. Chem.* **1994**, *59*, 7588−7590.

(35) A polyethyleneglycol−polystyrene based support (TentaGel) was used.

Scheme 5



stability toward typical TFA deprotection conditions widely used in peptide synthesis schemes was examined by incubating **28** with 95% TFA/5% H_2O for 1 h at room temperature. We were gratified to find that both the linker and thiazolidinone were stable as evidenced by complete retention of the benzylic resonance at 65 ppm (Figure 2b). Support **28** was also stable toward exposure to a standard TFA-scavenger cocktail containing phenol, thioanisole, water, and ethanedithiol for 2 h at room temperature (data not shown). Cleavage in PBS buffer containing 5% DMSO to simulate a biological assay medium was performed by irradiating a slurry of support **28** in a glass vial for several different times. The ^{13}C NMR spectra from 1.5 and 3 h of photolysis are shown in Figure 2, parts c and d, respectively, and represent roughly 60 and 95% cleavage. The liberated thiazolidinone was recovered in high yield (≈90%) and in high purity (95%) and was identical with authentic material as judged by HPLC and NMR analysis.

The support-bound photolysis is considerably slower than the corresponding solution photolysis due to several factors which include light scattering, shielding, or shadowing effects of the resin, and the swelling and solvation properties of the support. Estimates based on analysis via HPLC of the liberated compounds and NMR examination of the irradiated resin for the half life are 20−30 min in PBS, consistent with Figure 2D showing approximately 95% cleavage. Photolysis of resin **28** is significantly faster in organic solvents which parallels the observed solution kinetics, with 1−2 h of irradiation being sufficient to achieve high yields of liberated compound. To date we have employed linker **26** for the production of numerous small molecule amide libraries and have observed that basic scavengers such as hydrazine or ethanolamine (2−5 equiv) dramatically improves the rates of cleavage from the support. The use of less mass of resin also shortens the time required for complete cleavage, presumably due to less light scattering and shadowing effects. The hydroxyethyl linker **27** for the generation of small molecule acids also performs admirably, and its use and application will be reported in due course.

In conclusion we have described model studies leading to new *o*-nitrobenzyl cleavable linkers. Relying primarily on kinetic rates of cleavage to guide our selection process, we have developed the α-methyl-6-nitroveratryl-based chromaphore into linkers for solid phase organic synthesis. The addition of both a benzylic methyl group and incorporation of two alkoxy substituents to produce **26** and **27** dramatically enhances the cleavage rates upon photolysis at 365 nm relative to the previously known linker **4**. The new linkers are not so sensitive as to exclude their use in normal laboratory settings, however, and we are continuing to explore their use in combinatorial organic synthesis. The rapid cleavage kinetics in organic solvents and the ability to release compounds into biologically-relevant solvents now opens the way for high throughput screening techniques to employ linkers of this type.

Experimental Section

General. All melting points are uncorrected. Unless otherwise noted, materials were of the highest grade available from commercial sources and used without further purification. Commercial resin (TentaGel) was obtained from Rapp Polymere, Tübingen, Germany and was used without further characterization other than determination of the loading. Benzaldehyde (carbonyl-^{13}C) and Fmoc-glycine (2-^{13}C) were obtained from Cambridge Isotope Labs, Andover, MA. ^1H and ^{13}C NMR data were measured in the indicated solvent with tetramethylsilane as internal reference. Mass spectra were obtained with either APCI or ESI as ionization method. Combustion analyses were performed by the Microanalytical Laboratory, University of California at Berkeley.

***N*-Benzyl-4-(acetoxymethyl)-3-nitrobenzamide (4).** To a solution of 4-acetoxy-3-nitrobenzoic acid (1.02 g, 4.26 mmol), benzylamine (930 mg, 8.68 mmol), and 1-hydroxybenzotriazole hydrate (650 mg, 4.81 mmol) in 25 mL of DMF was added 1,3-dicyclohexylcarbodiimide (1.00g, 4.85 mmol), and the solution was stirred for 23 h. The reaction mixture was partitioned between EtOAc and saturated NaCl and was washed (saturated $NaHCO_3$, 1 N HCl), dried ($MgSO_4$), and evaporated to give a brown oil. Chromatography on silica gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2) afforded acetate **4** (0.81 g, 58% yield) as a light yellow oil which slowly solidified: mp 132−134 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.16 (s, 3 H), 4.63 (d, J = 6.3 Hz, 2 H), 5.51 (s, 2 H), 6.85 (br t, J = 6.3 Hz, 1 H), 7.24−7.37 (m, 5 H), 7.65 (d, J = 8.4 Hz, 1 H), 8.10 (dd, J = 8.4, 2.0 Hz, 1 H), 8.48 (d, J = 2.0 Hz, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.7, 44.3, 62.6, 123.4, 127.7, 127.9, 128.7, 129.1, 132.2, 135.0, 135.3, 137.5, 147.1, 164.6, 170.2; MS (APCI) m/z 329 $(MH)^+$. Anal. Calcd for $C_{17}H_{16}N_2O_5 \cdot 0.25H_2O$: C, 61.35; H, 5.00; N, 8.42. Found: C, 61.02; H, 5.02; N, 8.28.

***N*-Benzyl-(4-(2-bromopropionyl)phenoxy)acetamide (6).** To a solution of amide **5** (3.40 g, 14.1 mmol) and 2-bromopropionyl chloride (1.65 mL, 16.3 mmol) in 35 mL of carbon disulfide warmed to ≈40 °C was added aluminum trichloride (2.35 g, 17.6 mmol). The resultant black slurry was heated to reflux for 10 h and was then cooled to 0 °C and was quenched with water. The reaction mixture was partitioned between EtOAc and 1 N HCl, and the combined organic phase was washed (saturated NaCl), dried ($MgSO_4$), and evaporated to give the crude product as a white solid. Chromatography on silical gel (50% EtOAc/hexanes) afforded the product bromophenacyl amide **6** (4.52 g, 85% yield) as a white solid: mp 102−105 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.89 (d, J = 7.5 Hz, 3 H), 4.55 (d, J = 6.3 Hz, 2 H), 4.63 (s, 2 H), 5.24 (q, J = 7.5 Hz, 1 H), 6.80 (br t, 1 H), 6.96−7.02 (m, 2 H), 7.25−7.39 (m, 5 H), 7.99-8.07 (m, 2 H), a resonance for a minor isomer was observed: 1.73 (d, J = 7.5 Hz); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.1, 41.3, 43.1, 67.3, 114.6, 127.7, 128.2, 128.8, 131.4, 131.5, 137.5, 161.0, 167.1, 191.8; MS (APCI) m/z 378 $(MH)^+$, GCMS indicated the presence of ≈5% of the corresponding chloro derivative: 331 $(MH)^+$. Anal. Calcd for $C_{20}H_{18}NO_3Br$: C, 57.46; H, 4.82; N, 3.72. Found: C, 58.00; H, 5.09; N, 3.69. Repeated attempts to purify **6** to homogeneity were unsuccessful.

***N*-Benzyl-(4-(2-acetoxypropionyl)phenoxy)acetamide (7).** A solution of bromophenacyl amide **6** (500 mg, 1.33 mmol), HOAc (0.76 mL, 13.3 mmol), and diisopropylethylamine (2.31 mL, 13.3 mmol) was stirred at room temperature for 57 h. The reaction mixture was partitioned between EtOAc

and saturated NaCl, and the combined organic phase was dried over $MgSO_4$. Removal of the solvent under reduced pressure and chromatography of the residue on silical gel (50% EtOAc/hexanes) afforded pure acetate **7** (389 mg, 82% yield) as a white solid: mp 124−125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.52 (d, J = 7.6 Hz, 3 H), 2.14 (s, 3 H), 4.55, (d, J = 6.3 Hz, 2 H), 4.62 (s, 2 H), 5.92 (q, J = 7.6 Hz, 1 H), 6.81 (br t, J = 6.3 Hz, 1 H), 6.95−7.05 (m, 2 H), 7.25−7.38 (m, 5 H), 7.91−7.98 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 17.2, 20.7, 43.0, 67.2, 71.1, 114.6, 127.7, 128.5, 128.7, 130.9, 130.9, 137.5, 161.0, 167.1, 170.3, 195.1; MS (APCI) *m*/*z* 356 $(MH)^+$. Anal. Calcd for $C_{20}H_{21}NO_5 \cdot 0.05H_2O$: C, 67.42; H, 5.97; N, 3.43. Found: C, 67.11; H, 6.26; N, 3.87.

***tert*-Butyl (4-formyl-2-methoxyphenoxy)acetate (9a).** A slurry of vanillin (25.00 g, 164.3 mmol), *tert*-butyl bromoacetate (34.35 g, 176.1 mmol), and potassium carbonate (34.4 g, 249 mmol) was stirred at room temperature for 48 h. Water was added to the reaction mixture until all the salts had dissolved, and the mixture was partitioned between EtOAc and saturated NaCl acidified to pH 2 with 1 N HCl. The combined organic phase was dried ($MgSO_4$) and evaporated to give the aldehyde-ester **9a** (43.55 g, quantitative) as a white solid: mp 95−96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9 H), 3.95 (s, 3 H), 4.68 (s, 2 H), 6.86 (d, J = 8.4 Hz, 1 H), 7.40−7.46 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 27.8, 55.9, 65.9, 82.6, 109.6, 111.8, 125.9, 130.7, 149.7, 152.5, 166.8, 190.6; MS (GCMS) *m*/*z* 266 $(M)^+$. Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 63.05; H, 7.08.

***tert*-Butyl (4-Acetyl-2-methoxyphenoxy)acetate (9b).** A slurry of acetovanillone **8b** (10.50 g, 63.2 mmol), *tert*-butyl bromoacetate (13.50 g, 69.2 mmol), and K_2CO_3 (14.4 g, 104 mmol) in 75 mL of DMF was stirred at room temperature for 48 h. Water was added until all the salts were dissolved, and the reaction mixture was partitioned between EtOAc and saturated NaCl. The combined organic phase was washed (saturated NaCl), dried ($MgSO_4$), and evaporated to give the keto-ester **9b** (17.80 g, quantitative) as a white solid: mp 48−49 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (s, 9 H), 2.56 (s, 3 H), 3.94 (s, 3 H), 4.66 (s, 2 H), 6.78 (d, J= 8.3 Hz, 1 H), 7.50−7.56 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 26.1, 27.9, 55.9, 66.0, 82.6, 110.7, 111.6, 122.7, 131.3, 149.2, 151.4, 167.1, 196.6; MS (ESI) *m*/*z* 281 $(MH)^+$. Anal. Calcd for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.22; H, 7.28.

(4-Formyl-2-methoxy-5-nitrophenoxy)acetic Acid (10a). A solution of aldehyde-ester **9a** (5.00 g, 18.8 mmol) in 15 mL of acetic anhydride was added to a solution of 100 mL of 70% HNO_3 and 15 mL of acetic anhydride at 0 °C. The resultant orange solution was stirred for 2 h and then allowed to warm to room temperature, and stirring was continued for an additional 4 h. The reaction mixture was poured into water, the pH adjusted with solid NaOH to 3−4, and the solution saturated with solid NaCl. The aqueous phase was extracted with EtOAc and the combined organic phase dried ($MgSO_4$) and evaporated to give a yellow solid. Chromatography on silica gel (1% HOAc/5% MeOH/94% $CHCl_3$) gave pure aldehyde-acid **10a** (4.35 g, 91% yield) as a pale yellow solid: mp 163−164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.04 (s, 3 H), 4.82 (s, 2 H), 7.43 (s, 1 H), 7.57 (s, 1 H), 10.43 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 56.5, 65.4, 108.7, 110.5, 125.2, 143.2, 150.3, 152.6, 169.2, 188.5; MS (EI) *m*/*z* 255 $(M)^+$. Anal. Calcd for $C_{20}H_{22}N_2O_7 \cdot 0.2H_2O$: C, 46.41; H, 3.66; N, 5.41. Found: C, 46.16; H, 3.84; N, 5.72.

(4-Acetyl-2-methoxy-5-nitrophenoxy)acetic Acid (10b). A solution of keto-ester **9b** (5.06 g, 18.0 mmol) in 15 mL of acetic anhydride was added to a solution 15 mL of 70% HNO_3 and 10 mL of acetic anhydride cooled to 0 °C. The solution was stirred for 2 h and then allowed to warm to room temperature. After stirring an additional 4 h, the reaction mixture was poured into water and chilled to 4 °C overnight. The product was isolated by filtration, and the precipitate was washed extensively with water. Recrystalization from MeOH/H_2O afforded the product nitro-acid **10b** as light yellow crystals: mp 201−203 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3 H), 3.99 (s, 3 H), 4.75 (s, 2H), 6.69 (s, 1 H), 7.57 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + several drops of DMSO-d_6) δ 30.2, 56.5, 65.7, 108.5, 108.8, 133.4, 137.8, 147.6, 154.1, 169.4, 199.6; MS (APCI) *m*/*z* 270 $(MH)^+$. Anal. Calcd for $C_{11}H_{11}NO_7$: C, 49.08; H, 4.12; N, 5.20. Found: C, 48.84; H, 4.45; N, 5.10.

***N*-Benzyl-(4-formyl-2-methoxy-5-nitrophenoxy)acetamide (11a).** To a solution of aldehyde-acid **10a** (1.004 g, 3.934 mmol), benzylamine (0.850 mL, 7.78 mmol), and 1-hydroxybenzotriazole hydrate (1.06 g, 7.84 mmol) in 16 mL of DMF was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide (1.137 g, 5.931 mmol). The reaction mixture was stirred for 15 h and was then partitioned between EtOAc and saturated NaCl. The combined organic phase was washed (saturated $NaHCO_3$, 1 N HCl, saturated NaCl), dried ($MgSO_4$), and evaporated to give an off-white solid. Chromatography on silical gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2 to 15% acetone/CH_2Cl_2) to afford the product aldehyde-amide **11a** (0.97 g, 72% yield) as a light yellow solid: mp 176−178 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 3 H), 4.57 (d, J = 6.3 Hz, 2 H), 4.71 (s, 2 H), 7.02 (br t, 1 H), 7.27−7.40 (m, 5 H), 7.41 (s, 1 H), 7.65 (s, 1 H), 10.46 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 43.2, 56.7, 68.9, 110.3, 110.6, 127.7, 127.8, 128.8, 137.5, 143.4, 149.8, 153.7, 166.4, 187.4; MS (APCI) *m*/*z* 345 $(MH)^+$. Anal. Calcd for $C_{17}H_{16}N_2O_6$: C, 59.30; H, 4.68; N, 8.14. Found: C, 59.48; H, 5.08; N, 7.85.

***N*-Benzyl-(4-acetyl-2-methoxy-5-nitrophenoxy)acetamide (11b).** To a solution of nitro-acid **10b** (1.015 g, 3.770 mmol), benzylamine (0.825 mL, 7.55 mmol), and 1-hydroxybenzotriazole hydrate (562 mg, 4.16 mmol) in 25 mL of DMF was added 1,3-dicyclohexylcarbodiimide (880 mg, 4.26 mmol). The reaction mixture was stirred at room temperature for 27 h and was then partitioned between EtOAc and saturated NaCl. The organic phase was washed (saturated $NaHCO_3$, 1 N HCl), dried ($MgSO_4$), and evaporated to give a light yellow oil. Chromatography on silica gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2) gave pure keto-amide **11b** (0.80 g, 59% yield) as a light yellow solid: mp 138−140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.50 (s, 3 H), 3.86 (s, 3 H), 4.56 (d, J= 6.3 Hz, 2 H), 4.67 (s, 2 H), 6.75 (s, 1 H), 7.04 (br s, 1 H), 7.27−7.40 (m, 5 H), 7.68 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.4, 43.1, 56.5, 69.2, 109.2, 110.6, 127.7, 127.8, 128.8, 134.6, 137.5, 138.2, 147.2, 154.5, 166.7, 199.6; MS (APCI) *m*/*z* 359 $(MH)^+$. Anal. Calcd for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.14; H, 5.17; N, 7.83.

***N*-Benzyl-(4-(hydroxymethyl)-2-methoxy-5-nitrophenoxy)acetamide (12a).** To a solution of aldehyde-amide **11a** (0.28 g, 0.813 mmol) in 25 mL of THF was added $NaBH_4$ (80 mg, 2.11 mmol) at room temperature. The reaction mixture was stirred for 16 h and was then partitioned between EtOAc and saturated NH_4Cl. The combined organic phase was dried ($MgSO_4$) and evaporated to give a yellow residue. Chromatography on silica gel (1% MeOH/$CHCl_3$ to 5% MeOH/$CHCl_3$) afforded the alcohol-amide **12a** (63.6 mg, 23% yield) as a yellow solid: mp 177−188 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 3.90 (s, 3 H), 4.51 (d, J = 6.3 Hz, 2 H), 4.64 (s, 2 H), 4.99 (d, J = 6.3 Hz, 2 H), 5.10 (t, J = 6.3 Hz, 1 H), 7.23−7.38 (m, 5 H), 7.47 (s, 1 H),, 7.65 br t, 1 H), 7.77 (s, 1H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 41.9, 56.1, 60.1, 68.1, 110.0, 110.3, 126.8, 127.2, 128.2, 135.8, 138.1, 139.1, 145.4, 153.9, 167.2; MS (APCI) 347 $(MH)^+$. Anal. Calcd for $C_{17}H_{16}N_2O_6$: C, 58.96; H, 5.24; N, 8.09. Found: C, 58.84; H, 5.22; N, 8.12.

***N*-Benzyl-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)acetamide (12b).** To a solution of keto-amide **11b** (207 mg, 0.578 mmol) in 20 mL of THF was added $NaBH_4$ (78 mg, 2.06 mmol) at room temperature. The reaction mixture was stirred for 31 h and was then partitioned between EtOAc and saturated NH_4Cl. The combined organic phase was dried over $MgSO_4$ and evaporated to give a yellow solid. Chromatography on silica gel (5% MeOH/$CHCl_3$) afforded pure alcohol **12b** (210 mg, 97% yield) as a yellow solid: mp 152−153 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (d, J = 7.1 Hz, 3 H), 2.27 (br d, J = 2.5 Hz, 1 H), 3.85 (s, 3 H), 4.55 (d, J = 6.3 Hz, 2 H), 4.63 (s, 2H), 5.59 (dq, J = 7.1, 2.5 Hz, 1 H), 7.14 (br t, 1 H), 7.26 (s, 1 H), 7.27−7.39 (m, 5 H), 7.63 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$ + several drops of DMSO-d_6) δ 24.7, 42.6, 55.8, 64.5, 69.1, 109.2, 111.3, 127.1, 127.2, 128.3, 137.5, 138.7, 140.1, 144.9, 153.8, 167.1; MS (APCI) m/*z* 361 $(MH)^+$. Anal. Calcd

for $C_{18}H_{20}N_2O_6$: C, 59.99; H, 5.59; N, 7.77. Found: C, 59.97; H, 5.68; N, 7.52.

***N*-Benzyl-(4-(acetoxymethyl)-2-methoxy-5-nitrophenoxy)acetamide (13a).** To a solution of aldehyde-amide **11a** (74.5 mg, 0.216 mmol) in 10 mL of THF was added $NaBH_4$ (75 mg, 1.98 mmol) at room temperature. After stirring for 1 h, the reaction was quenched with excess glacial acetic acid until all gas evolution had ceased. Pyridine (1 mL) and acetic anhydride (0.5 mL, 5.3 mmol) were added, and the solution was stirred an additional 22 h. The reaction mixture was partitioned between EtOAc and saturated NaCl acidified to pH 2 with 1 N HCl. The combined organic phase was evaporated to dryness to give crude acetate-amide **13a** as a light yellow solid. Chromatography on silica gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2) afforded pure acetate-amide **13a** (58.3 mg, 70% overall yield for both steps): mp 128−131 °C; ^{1}H NMR (300 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 2.17 (s, 3 H), 3.85 (s, 3 H), 4.55 (d, J = 6.3 Hz, 2 H), 4.64 (s, 2 H), 5.50 (s, 2 H), 7.01 (s, 1 H), 7.11 (br t, 1 H), 7.27−7.40 (m, 5 H), 7.76 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.8, 43.0, 56.1, 63.0, 69.4, 111.0, 112.3, 127.6, 127.7, 128.7, 128.9, 137.6, 139.8, 145.9, 154.0, 167.1, 170.1; MS (APCI) *m*/*z* 389 $(MH)^+$. Anal. Calcd for $C_{19}H_{20}N_2O_7$: C, 58.76; H, 5.19; N, 7.21. Found: C, 58.78; H, 5.04; N, 7.19.

***N*-Benzyl-(4-(1-acetoxyethyl)-2-methoxy-5-nitrophenoxy)acetamide (13b).** A mixture of alcohol **12b** (112 mg, 0.311 mmol), pyridine (1 mL), acetic anhydride (0.5 mL), and DMAP (5 mg) in 10 mL of CH_2Cl_2 was stirred for 48 h. The reaction mixture was partitioned between EtOAc and saturated NaCl, and the organic phase was dried (MgSO4) and evaporated. The crude product was chromatographed on silica gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2) to afford pure acetate **13b** (105.8 mg, 85% yield) as a pale yellow solid: mp 115−116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (d, J = 6.7 Hz, 3 H), 2.08 (s, 3 H), 3.84 (s, 3 H), 4.55 (d, J = 6.3 Hz, 2 H), 4.62 (s, 2 H), 6.46 (q, J = 6.7 Hz, 1 H), 7.03 (s, 1 H), 7.14 (br t, 1 H), 7.25−7.39 (m, 5 H), 7.63 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.1, 21.9, 43.0, 56.1, 68.1, 69.4, 108.6, 111.8, 127.6, 127.7, 128.7, 135.2, 137.6, 139.7, 145.7, 154.1, 167.2, 169.5; MS (APCI) *m*/*z* 403 $(MH)^+$. Anal. Calcd for $C_{20}H_{22}N_2O_7$: C, 59.70; H, 5.51; N, 6.96. Found: C, 59.58; H, 5.56; N, 7.03.

Methyl 4-(4-Acetyl-2-methoxyphenoxy)butanoate (14). A slurry of acetovanillone (41.00 g, 246.7 mmol), methyl 4-bromobutyrate (49.63 g, 274.1 mmol), and K_2CO_3 (51.1 g, 370 mmol) in 200 mL of DMF was stirred at room temperature for 16 h. Water was added to the reaction mixture until all the salts were dissolved, and the solution was partitioned between EtOAc and saturated NaCl. The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness to afford 67.90 g (100% crude yield) of product keto-ester **14** as a colorless oil which slowly solidified: mp 48−49 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.19 (pentet, J = 7.3 Hz, 2 H), 2.56 (t, J = 7.3 Hz, 2 H), 2.56 (s, 3 H), 3.70 (s, 3 H), 3.91 (s, 3 H), 4.15 (t, J = 7.3 Hz, 2 H), 6.89 (d, J = 8.4 Hz, 1 H), 7.51−7.78 (m, 2 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.2, 26.1, 30.2, 51.5, 55.9, 67.6, 110.4, 111.2, 123.1, 130.4, 149.2, 152.5, 173.3, 196.7; MS (EI) *m*/*z* 266 (M^+). Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.15; H, 6.81. Found: C, 62.81; H, 6.83.

Methyl 4-(4-Acetyl-2-methoxy-5-nitrophenoxy)butanoate (15). A solution of keto-ester **14** (5.00 g, 18.8 mmol) in 15 mL of acetic anhydride was slowly added to a solution of 100 mL of 70% HNO_3 and 20 mL of acetic anhydride at 0 °C. After stirring for 2.5 h the reaction mixture was poured into water and chilled to 4 °C overnight. The precipitate was collected by filtration and washed extensively with water. Recrystalization from MeOH/H_2O afforded the nitro-ester **15** as yellow crystals (3.87 g, 66% yield): mp 112−113 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (pentet, J = 7.1 Hz, 2 H), 2.49 (s, 3 H), 2.56 (t, J = 7.1 Hz, 1 H), 3.70 (s, 3 H), 3.95 (s, 3 H), 4.15 (t, J = 7.1 Hz, 2 H), 6.75 (s, 1 H), 7.67 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.1, 30.2, 30.4, 51.7, 56.6, 68.4, 108.0, 108.7, 132.8, 138.2, 148.8, 154.3, 173.2, 200.0; MS (EI) *m*/*z* 311 (M^+), 280. Anal. Calcd for $C_{14}H_{17}NO_7$: C, 54.02; H, 5.50; N, 4.50. Found: C, 53.98; H, 5.60; N, 4.49.

4-(4-Acetyl-2-methoxy-5-nitrophenoxy)butanoic Acid (16). A solution of nitro-ester **15** (2.44 g, 7.84 mmol) and 15 mL (15 mmol) of 1 N NaOH in 100 mL of MeOH were stirred at room temperature for 21 h before being partitioned between EtOAc and sat NaCl acidified to pH 2 with 1 N HCl. The organic phase was washed (saturated NaCl), dried ($MgSO_4$), and evaporated to dryness. The crude product was chromatographed on silica gel (1% HOAc/1% MeOH/98% $CHCl_3$ to 1% HOAc/5% MeOH/94% $CHCl_3$) to afford recovered **15** (0.78 g) and pure acid **16** as a yellow solid (1.55 g, 97% yield based on recovered **15**): mp 159−160 °C; ^{1}H NMR (300 MHz, $CDCl_3$ + 2 drops of DMSO-d_6) δ 2.17 (pentet, J = 6.7 Hz, 2 H), 2.50 (s, 3 H), 2.53 (t, J = 6.7 Hz, 2 H), 3.97, (s, 3H), 4.18 (t, J = 6.7 Hz, 2 H), 6.78 (s, 1 H), 7.63 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 23.9, 29.9, 30.0, 56.6, 68.2, 108.0, 109.8, 131.1, 138.3, 148.5, 153.3, 173.9, 199.2; MS (EI) *m*/*z* 297 (M^+), 196. Anal. Calcd for $C_{13}H_{15}NO_7$: C, 52.53; H, 5.09; N, 4.71. Found: C, 52.49; H, 5.09; N, 4.61.

***N*-Benzyl-4-(4-acetyl-2-methoxy-5-nitrophenoxy)butyramide (17).** To a solution of acid **16** (503 mg, 1.69 mmol), benzylamine (0.37 mL, 3.39 mmol), and 1-hydroxybenzotriazole hydrate (459 mg, 3.39 mmol) in 8 mL of DMF was added 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide methiodide (542 mg, 2.83 mmol). The reaction mixture was stirred for 15 h and was then partitioned between EtOAc and saturated NaCl. The combined organic phase was washed (1 N HCl, saturated $NaHCO_3$), dried ($MgSO_4$), and evaporated to give a white solid. Chromatography on silical gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2 to 15% acetone/CH_2Cl_2) afforded the product amide **17** (0.58 g, 89% yield) as a white solid: mp 142−143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.25 (pentet, J = 7.1 Hz, 2 H), 2.46 (t, J = 7.1 Hz, 2 H), 2.48 (s, 3 H), 3.89 (s, 3 H), 4.15 (t, J = 7.1 Hz, 2 H), 4.44 (d, J = 6.3 Hz, 2 H), 5.93 (br t, 1 H), 6.72 (s, 1 H), 7.21−7.35 (m, 5 H), 7.59 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.7, 30.3, 32.5, 43.6, 56.5, 68.5, 108.0, 108.7, 127.5, 127.7, 128.7, 132.8, 138.1, 138.4, 148.7, 154.1, 171.6, 200.0; MS (ESI) *m*/*z* 387 (MH^+), 409 $(M + Na)^+$. Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.16; H, 5.73; N, 7.22.

***N*-Benzyl-4-(4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy)butyramide (18).** To a solution of **17** (397 mg, 1.03 mmol) in 20 mL of THF was added $NaBH_4$ (83 mg, 2.19 mmol) at room temperature. The reaction mixture was stirred for 26 h and was then partitioned between EtOAc and saturated NH_4Cl. The combined organic phases were dried ($MgSO_4$) and evaporated to give crude alcohol. Chromatography on silica gel (1% MeOH/$CHCl_3$ to 5% MeOH/$CHCl_3$) gave alcohol **18** (398 mg, 100% yield) as a light yellow solid: mp 124−125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (d, J = 6.3 Hz, 3 H), 2.21 (pentet, J = 6.7 Hz, 2 H), 2.46 (t, J = 6.7 Hz, 2 H), 2.63 (br s, 1 H), 3.87 (s, 3 H), 4.09 (t, J = 6.7 Hz, 2 H), 4.42 (d, J = 6.3 Hz, 2 H), 5.55 (dq, J = 6.3, 2.9 Hz, 1 H), 6.08 (br t, J = 6.3 Hz, 1 H), 7.20−7.33 (m, 6 H), 7.53 (s, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 24.7, 25.2, 31.5, 42.0, 56.0, 63.9, 68.2, 108.3, 109.1, 126.7, 127.1, 128.2, 138.0, 138.9, 139.6, 146.2, 153.4, 171.4; MS (ESI) *m*/*z* 389 (MH^+), 411 $(M + Na)^+$. Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.85; H, 6.23; N, 7.21. Found: C, 62.01; H, 6.24; N, 7.21.

***N*-Benzyl-4-(4-(1-acetoxyethyl)-2-methoxy-5-nitrophenoxy)butyramide (19).** A solution of alcohol **18** (145 mg, 0.373 mmol) and 1 mL of pyridine in 10 mL of CH_2Cl_2 was treated with acetic anhydride (0.5 mL, 5.3 mmol) and catalytic DMAP for 4 h. The reaction mixture was partitioned between EtOAc and saturated NaCl, washed (1 N HCl), dried ($MgSO_4$), and evaporated to give crude acetate product as a pale yellow solid. Chromatography on silica gel (CH_2Cl_2 to 5% acetone/CH_2Cl_2) gave pure acetate **19** (170.0 mg, quantitative) as a pale yellow solid: mp 114−115 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.61 (d, J = 6.7 Hz, 3 H), 2.07 (s, 3 H), 2.22 (pentet, J = 7.0 Hz, 2 H), 2.45 (t, J = 7.0 Hz, 2 H), 3.88 (s, 3 H), 4.10 (t, J = 7.0 Hz, 2 H), 4.43 (d, J = 6.3 Hz, 2 H), 6.04 (br t, 1 H), 6.46 (q, J = 6.7 Hz, 1 H), 6.98 (s, 1 H), 7.20−7.33 (m, 5 H), 7.55 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 21.1, 22.0. 24.8, 32.8, 43.6, 56.2, 68.3, 68.3, 108.1, 109.0, 127.5, 127.7, 128.6, 133.2, 138.2, 139.8, 147.1, 153.8, 169.6, 171.8; MS (ESI) *m*/*z* 431 $(MH)^+$. Anal. Calcd for $C_{22}H_{26}N_2O_7$: C, 61.39; H, 6.09; N, 6.51. Found: C, 61.47; H, 6.25; N, 6.59.

4-(4-(1-Acetamidoethyl)-2-methoxy-5-nitrophenoxy)-butanoic Acid (20). To a solution of Fmoc-linker **26** (217 mg, 0.417 mmol) in 35 mL of MeOH and 10 mL of water was added 1 N NaOH (6.0 mL, 0.60 mmol). The solution was stirred for 16 h before being concentrated under reduced pressure to 5−10 mL. *p*-Dioxane (35 mL) and water (10 mL) were added to the resultant slurry, and the pH was adjusted to 7−8 with 6 N HCl. Acetic anhydride (2 mL) was added, and the solution was stirred at room temperature for 18 h. The reaction mixture was partitioned between Et_2O (washings discarded) and saturated $NaHCO_3$. The aqueous phase was acidified to pH 1 with 1 N HCl and extracted with EtOAc. The combined organic phase was dried ($MgSO_4$) and evaporated to afford the crude acetate product as a tan oil. Chromatography on silical gel (1% HOAc/15% MeOH/$CHCl_3$) gave pure acetate-acid **20** (125.0 mg, 88% yield) as a yellow solid: mp 188−192 °C; ^{1}H NMR (300 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 1.48 (d, J = 7.1 Hz, 3 H), 1.95 (s, 3 H), 2.13 (pentet, J = 7.1 Hz, 2 H), 2.49 (t, J = 7.1 Hz, 2 H), 3.93 (s, 3 H), 4.10 (t, J = 7.1 Hz, 2 H), 5.59 (dq, J = 7.1, 7.1 Hz, 1 H), 7.09 (s, 1 H), 7.53 (s, 1 H), 7.70 (d, J = 7.1 Hz, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 21.3, 22.6, 23.9, 30.0, 45.5, 56.0, 67.9, 109.0, 109.5, 134.8, 139.9, 146.3, 153.4, 169.2, 174.9; MS (APCI) *m*/*z* 341 $(MH)^+$. Anal. Calcd for $C_{15}H_{20}N_2O_7 \cdot 0.1H_2O$: C, 52.66; H, 5.95; N, 8.19. Found: C, 52.35; H, 5.95; N, 7.89.

***N*-Benzyl-4-(4-(1-acetamidoethyl)-2-methoxy-5-nitrophenoxy)butyramide (21).** To a solution of acetate-acid **20** (83.2 mg, 0.244 mmol), benzylamine (80 μL, 0.732 mmol), and 1-hydroxybenzotriazole hydrate (66 mg, 0.488 mmol) in 3 mL of DMF was added 1,3-diisopropylcarbodiimide (40 μL, 0.255 mmol). The reaction mixture was stirred at room temperature for 22 h and was then partitioned between EtOAc and saturated NaCl. The organic phase was washed (1 N HCl, saturated $NaHCO_3$, sat NaCl), dried ($MgSO_4$), and evaporated to give a light yellow oil. Chromatography on silica gel (50% acetonitrile/CH_2Cl_2 to 2% HOAc/49% acetonitrile/49% CH_2Cl_2) afforded amide **21** (75.7 mg, 72% yield) as a light yellow solid: mp 202−204 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.53 (d, J = 7.0 Hz, 3 H), 1.98 (s, 3 H), 2,21 (pentet, J = 7.0 Hz, 2 H), 2.45 (t, J = 7.0 Hz, 2 H), 3.86 (s, 3 H), 4.10 (t, J = 7.0 Hz, 2 H), 4.44 (d, J = 6.3 Hz, 2 H), 5.47 (dq, J = 7.0, 7.0 Hz, 1 H), 5.98 (br t, 1 H), 6.39 (br d, J = 7.0 Hz, 1 H), 6.87 (s, 1 H), 7.21−7.34 (m, 5 H), 7.53 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 21.3, 22.6, 24.6, 32.2, 43.0, 45.6, 55.9, 68.3, 109.2, 109.6, 126.8, 127.3, 128.1, 134.8, 138.4, 139.8, 146.3, 153.7, 169.1, 171.8; MS (ESI) *m*/*z* 430 $(MH)^+$. Anal. Calcd for $C_{22}H_{27}N_3O_6 \cdot 0.3H_2O$: C, 60.76; H, 6.40; N, 9.66. Found: C, 60.40; H, 6.42; N, 9.26.

Methyl 4-(4-(1-(Hydroxyimino)ethyl)-2-methoxyphenoxy)butanoate (22). To a solution of the keto-ester **14** (68.4 g) in 225 mL of 2:1 pyridine:H_2O was added hydroxylamine hydrochloride (21.46 g, 309 mmol). After stirring at room temperature for 14 h the reaction mixture was partitioned between EtOAc and saturated NaCl. The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness to afford oxime **22** (69.94 g, 100% crude yield) as a white solid: mp 82−83 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (pentet, J = 8.0 Hz, 2 H), 2.26 (s, 3 H), 2.55 (t, J = 8.0 Hz, 2 H), 3.68 (s, 3 H), 3.88 (s, 3 H), 4.09 (t, J = 8.0 Hz, 2 H), 6.87 (d, J = 8.8 Hz, 1 H), 7.12 (dd, J = 2.1, 8.8 Hz, 1 H), 7.25 (d, J = 2.1 Hz, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 11.9, 24.4, 30.4, 51.6, 55.9, 67.8, 109.0, 112.4, 119.1, 129.5, 149.3, 149.4, 155.6, 173.6; MS (APCI) *m*/*z* 282 (MH^+). Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98. Found: C, 59.62; H, 6.75; N, 4.81.

Methyl 4-(2-Methoxy-4-(1-(trifluoroacetamido)ethyl)-phenoxy)butanoate (23). A slurry of the oxime **22** from above (146.7 mmol) and 10% palladium on charcoal (2.5 g) in 400 mL of glacial acetic acid was degassed twice by placing the flask under reduced pressure with an aspirator and subsequently filling the flask with H_2. The reaction mixture was placed under 1.1 atm of hydrogen gas via a balloon and vigorously stirred at room temperature. An additional 2 g of catalyst was added after 18 h, and the balloon was refilled with hydrogen as the gas was consumed. An additional 2 g of catalyst was added after 2 days. The reaction mixture was filtered after 5 days, and the solvent was removed under vacuum. The oily residue was taken up in 600 mL of water and acidified to pH 1 with 6 N HCl. The aqueous phase was extracted with Et_2O (washings discarded), and the aqueous phase was basified with solid NaOH to pH 11 and extracted with EtOAc. The EtOAc phase was dried ($MgSO_4$), filtered, and evaporated to dryness to afford the intermediate amine as a colorless oil.

The crude amine was taken up in 300 mL of pyridine, cooled to 0 °C with an ice bath, and treated with trifluoroacetic anhydride (31 mL, 219 mmol) for 1 h. The reaction mixture was worked up by partitioning between EtOAc and saturated NaCl. The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness to give the crude trifluoroacetate as a light yellow solid. The solid was recrystallized from CH_2Cl_2/hexanes to afford trifluoroacetate **23** (71.62 g, 80% overall yield from acetovanillone) as a white solid: mp 96−97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.58 (d, *J* = 7.5 Hz, 3 H), 2.15 (pentet, *J* = 7.1 Hz, 2 H), 2.54 (t, *J* = 7.1 Hz, 2 H), 3.68 (s, 3 H), 3.86 (s, 3 H), 4.05 (t, *J* = 7.1 Hz, 2 H), 5.09, (dq, *J* = 7.5, 7.5 Hz, 1 H), 6.38 (br d, *J* = 7.5 Hz, 1 H), 6.80−6.90 (m, 3 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.8, 24.4, 30.4, 49.5, 51.6, 56.0, 67.9, 110.4, 113.4, 118.2, 133.7, 148.2, 149.7, 173.6, (the resonances for N*C*OCF$_3$ and NCO*C*F$_3$ were not observed); MS (APCI) *m*/*z* 364 (MH^+), 251 $(MH-H_2NCOCF_3)^+$. Anal. Calcd for $C_{16}H_{20}F_3NO_5$: C, 52.89; H, 5.55; N, 3.86. Found: C, 52.76; H, 5.45; N, 3.59.

Methyl 4-(2-Methoxy-5-nitro-4-(1-(trifluoroacetamido)-ethyl)phenoxy)butanoate (24). Trifluoroacetate **23** (9.40 g, 25.9 mmol) was slowly added to 200 mL of 70% HNO_3 cooled to 0 °C. The solution turned orange in color and was quenched after 2 h by pouring into water and adjusting the total volume to 2 L. The resultant slurry was chilled to 4 °C overnight and filtered to give a light yellow solid. The solid was washed with water and recrystallized from MeOH/H_2O to afford the nitrated acetate **24** (9.07 g, 86% yield) as a light yellow solid: mp 156−157 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62 (d, *J* = 7.9 Hz, 3 H), 2.18 (pentet, *J* = 7.5 Hz, 2 H), 3.70 (s, 3 H), 3.94 (s, 3 H), 4.12 (t, *J* = 7.5 Hz, 2 H), 5.50 (dq, *J* = 7.9, 7.9 Hz, 1 H), 6.87 (s, 1 H), 7.37 (br d, *J* = 7.9 Hz, 1 H), 7.61 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.1, 24.2, 30.3, 48.4, 51.7, 56.4, 68.2, 110.2, 111.0, 130.9, 140.5, 147.6, 154.0, 156.6, 173.3, the resonance for NCO*C*F$_3$ was not observed; MS (APCI) *m*/*z* 409 (MH^+). Anal. Calcd for $C_{16}H_{19}F_3N_2O_7 \cdot 0.05H_2O$: C, 46.96; H, 4.70; N, 6.85. Found: C, 46.59; H, 4.78; N, 6.89.

4-(4-(1-(9-Fluorenylmethoxycarbonylamino)ethyl)-2-methoxy-5-nitrophenoxy)butanoic Acid (26). To a solution of the nitrated compound **24** (12.36 g, 30.27 mmol) in 250 mL of warm MeOH was added 1 N NaOH (100 mL, 100 mmol), and the resultant solution was heated to reflux for 5 h. The solution was cooled to room temperature and concentrated to approximately 100 mL with a rotary evaporator. *p*-Dioxane (150 mL) and H_2O (100 mL) were added, and the pH of the solution was adjusted to pH 9 with 6 N HCl. A solution of Fmoc-Cl (9.83 g, 38.0 mmol) in 100 mL of dioxane was added, and an additional 25 mL of dioxane was added to create a homogeneous solution. The pH of the solution was adjusted with 1 N NaOH to pH 8 over the next 30 min, and a light yellow precipitate formed during this time. The reaction was quenched after 18 h by adding 100 mL of 1 N HCl and adjusting the total volume to 1 L with H_2O. The precipitate was collected, taken up in 1 L of hot EtOAc, dried over $MgSO_4$, and filtered while hot. The solvent was removed under reduced pressure, affording a light yellow solid which was triturated with 1 L of hot Et_2O. The solid was collected and was recrystallized from MeOH to afford the Fmoc linker **26** (12.78 g, 81% yield for two steps) as a light yellow solid: mp 200−201 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.43 (d, *J* = 7.4 Hz, 3 H), 1.95 (pentet, *J* = 7.3 Hz, 2 H), 2.39 (t, *J* = 7.3 Hz, 2 H), 3.87 (s, 3 H), 4.05 (t, *J* = 7.3 Hz, 2 H), 4.16 (t, *J* = 6.9 Hz, 1 H), 4.27 (m, 2 H), 5.19 (dq, *J* = 7.4 Hz, 1 H), 7.25 (s, 1 H), 7.29 (t, *J* = 7.3 Hz, 2 H), 7.39 (t, *J* = 7.3 Hz, 2 H), 7.48 (s, 1 H), 7.64 (d, *J* = 8.3 Hz, 2 H), 7.87 (d, *J* = 8.3 Hz, 2 H), 8.06 (d, *J* = 8.3 Hz, 1 H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 21.9, 24.0, 29.9, 46.0, 46.7, 56.2, 65.2, 67.9, 108.2, 109.4, 120.1, 125.0, 126.9, 127.6, 135.5, 139.9, 140.7, 143.6, 143.9, 146.3, 153.4,

155.3, 174.0; MS (APCI) *m/z* 551 (MH^+), 282, 179. Anal. Calcd for $C_{28}H_{28}N_2O_8 \cdot 0.3H_2O$: C, 63.94; H, 5.48; N, 5.33. Found: C, 63.57; H, 5.44; N, 5.54.

4-(4-(1-Hydroxyethyl)-2-methoxy-5-nitrophenoxy)butanoic Acid (27). To a solution of ester **15** (24.19 g, 77.72 mmol) in 400 mL of THF and 200 mL of MeOH was added $NaBH_4$ (3.61 g, 95.4 mmol) at room temperature. An additional 1 g (26.4 mol) of $NaBH_4$ was added after stirring for 1 h. The reaction mixture was stirred for 15 h at room temperature by which time TLC indicated that complete reduction had taken place. 1 N NaOH (200 mL, 0.200 mmol) and H_2O (100 mL) were added and the reaction stirred 7 h. The reaction mixture was concentrated under reduced pressure to approximately 400 mL, carefully acidified with 6 N HCl, and then extracted with EtOAc. The combined organic phase was dried ($MgSO_4$) and evaporated to give a yellow residue. Recrystallization from EtOAc/hexane afforded photolinker **27** (16.61 g, 71% yield) as a pale yellow solid: mp 164−168 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 1.49 (d, J = 7.0 Hz, 2 H), 2.15 (pentet, J = 7.0 Hz, 2 H), 2.52 (t, J = 7.0 Hz, 2 H), 3.98 (s, 3 H), 4.13 (t, J = 7.0 Hz, 2 H), 4.60 (bs, 1 H), 5.53 (q, J = 7.0 Hz, 1 H), 7.40 (s, 1 H), 7.57 (s, 1 H); ^{13}C NMR (75.5 MHz, $CDCl_3$ plus several drops of DMSO-d_6) δ 23.9, 24.7, 30.0, 56.0, 64.6, 68.0, 108.5, 108.7, 138.3, 138.8, 146.2, 153.8, 174.6; MS (APCI) 300 $(MH)^+$. Anal. Calcd for $C_{13}H_{17}NO_7$: C, 52.17; H,, 5.73; N, 4.68. Found: C, 52.50; H, 5.63; N, 4.72.

4-Thiazolidinone−Photolinker−Glycine−TentaGel (28). TentaGel-S-NH_2 (0.50 g, 0.30 mmol/g loading) was washed with DMF, and a 0.25 M solution of Fmoc-glycine (2-^{13}C, 99%) symmetrical anhydride (prepared from 182 mg of Fmoc-Gly-OH and 50 μL of DIC in 1.2 mL of DMF) was coupled to the resin for 1 h, by which time ninhydrin had revealed that a complete reaction had taken place. The resin was washed with DMF, capped with 20% Ac_2O, 30% pyridine, and 50% CH_2Cl_2 for 30 min, and washed with CH_2Cl_2, DMF, and MeOH. The Fmoc group was removed by incubating the resin in 30% piperidine/DMF for 30 min, followed by washing with DMF. The resin was treated with a 0.20 M solution of OBt-activated Fmoc-photolinker **26** (prepared from 310 mg of **26**, 92 mg of HOBt, 95 μL of DIC in 3 mL of DMF) for 16 h. Ninhydrin test indicated a complete reaction had taken place. The resin was washed with DMF and CH_2Cl_2 and was then capped with 20% Ac_2O, 30% pyridine, and 50% CH_2Cl_2 for 30 min, followed by washing with DMF and CH_2Cl_2. The resin was deprotected with 30% piperidine/DMF for 30 min and then washed with DMF. A 0.5 M solution of Fmoc-glycine symmetrical anhydride (prepared from 182 mg of Fmoc-Gly-OH and 50 μL of DIC in 0.6 mL of DMF) was coupled to the resin for 1 h followed by washing as before. Deprotection of the Fmoc group with piperidine, washing, and drying as above gave roughly 150 mg of dry resin. A portion of the resin (40 mg) was transferred into a 4-mL vial, acetonitrile (2 mL), 3 Å molecular sieves (20−30 pellets), benzaldehyde (carbonyl-^{13}C, 99%) (152 μL), and mercaptoacetic acid (300 μL) were added, and the vial was heated to 70 °C for 2 h. The resin was transferred to a disposable filter tube and washed extensively (3 × 5 mL of CH_2Cl_2, 3 × 5 mL of DMF, 3 × 5 mL of CH_2Cl_2, 3 × 5 mL of MeOH, 3 × 5 mL of CH_2Cl_2, 3 × 5 mL of Et_2O).

General Photolysis Conditions. Solution photolyses were conducted with a 1000 W Hg(Xe) ARC lamp fitted with a water filter and a 350−450 nm dichroic reflector on a horizontal axis. The output was passed through a collimating lens (Oriel Corp.) and the sample (0.10 mM) irradiated by placing a quartz 0.2 cm path length cuvette in the beam for various times. The power level at the cuvette was adjusted to 10 mW/cm^2 as determined with a Optical Associates Inc. UV powermeter with a 365 nm wavelength probe. The percent remaining starting material was determined via reverse-phase C_{18} HPLC analysis of the photolyzed solutions with 220 nm detection.

Resin photolyses were conducted with 50 mg of resin suspended in 1−2 mL of pH 7.4 PBS buffer containing 5% of DMSO. Photolyses were conducted by irradiating the samples with a 500 W Hg ARC lamp fitted with a 350−450 nm dichroic mirror on a vertical axis at a 10 mW/cm^2 power level measured at 365 nm. The samples were irradiated from above with gentle mixing from an orbital shaker table. After photolysis the supernatant was analyzed by reverse-phase HPLC and the resin analyzed via gel phase ^{13}C NMR for the disappearance of the thiazolidinone resonance.

Stability of 28 Toward TFA Treatment. A portion of resin **28** was treated with 95% TFA/5% H_2O for 1 h, followed by washing with CH_2Cl_2, MeOH, and Et_2O. In a separate experiment, an additional 20 mg of resin was treated for 2 h at room temperature with a solution of phenol (75 mg), thioanisole (50 μL), water (50 μL), ethanedithiol (25 μL), and TFA (1 mL) followed by washing as above. Gel ^{13}C NMR analysis of the resin indicated no loss of thiazolidinone or photolabile linker, as evidenced by the relative integration of the two labeled carbons.

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