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Solid-Phase Synthesis of Carboxylic and Oxamic Acids via $OsO_4/NaIO_4$/HMTA-Mediated Oxidative Cleavage of Acetylenic Peptides

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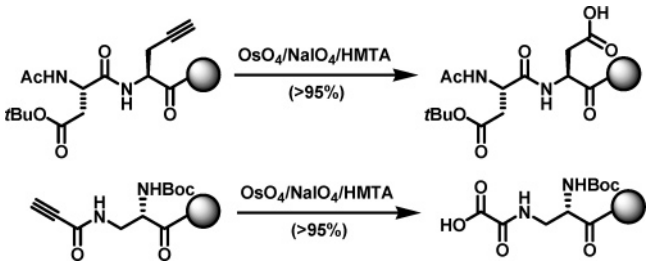
ABSTRACT



A general method for the solid-phase synthesis of carboxy-functionalized peptides by oxidative cleavage of alkynes is presented. Clean and quantitative conversion is enabled by the addition of bases, such as DABCO and HMTA, to the classical $OsO_4/NaIO_4$ mixture. The utility of the reaction is further illustrated by the synthesis of oxamic acids.

Since the pioneering work of Merrifield,[1] solid-phase synthesis has emerged as a powerful approach for small- and large-scale production of peptides and peptidomimetics. With more than 200 new peptide-based drugs under different stages of development,[2] where approximately half of these are estimated to be in clinical trials or prior to approval, efficient synthetic methodology for the high-throughput generation of novel peptides and peptidomimetics is as relevant as ever to satisfy the demands of the rapidly growing market of therapeutic peptides.[3]

During the years, massive experimental efforts have focused on providing reliable coupling reagents and additives for activating the carboxylic acid moiety for subsequent coupling with amino residues.[4] In combination with numerous possibilities for varying the protecting group strategy, the linker, and the resin, most peptide sequences may now routinely be made on the solid support in high purities.[5] However, proportionally little time has been spent on developing solid-phase synthetic methodology for site-selective quantitative introduction of functional groups onto readily available peptide frameworks without resorting to cumbersome protecting group strategies.[6] Such functional groups could represent synthetic end-points, e.g., as pharmacophores and biasing elements, or serve as handles for further synthetic manipulations.

As part of our work on solid-supported peptide aldehydes,[7] we have previously reported how solid-supported aldehydes may be cleanly generated by $OsO_4/NaIO_4$/DABCO-mediated oxidative cleavage of alkenes.[8] A key discovery in these

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(2) Bruckdorfer, T.; Marder, O.; Albericio, F. *Curr. Pharm. Biotechnol.* **2004**, *5*, 29−43.
(3) Loffet, A. *J. Peptide Sci.* **2002**, *8*, 1−7.
(4) For recent reviews on coupling reagents, see: (a) Montalbetti, C. A. G. N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827−10852. (b) Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447−2467.
(5) Jung, G. *Combinatorial Peptide and Nonpeptide Libraries*; Wiley-VCH: Weinheim, 1996.
(6) For an overview of solid-phase synthesis: Dörwald, F. Z. *Organic Synthesis on Solid Phase: Supports, Linkers, Reactions*; Wiley-VCH: Weinheim, 2002.

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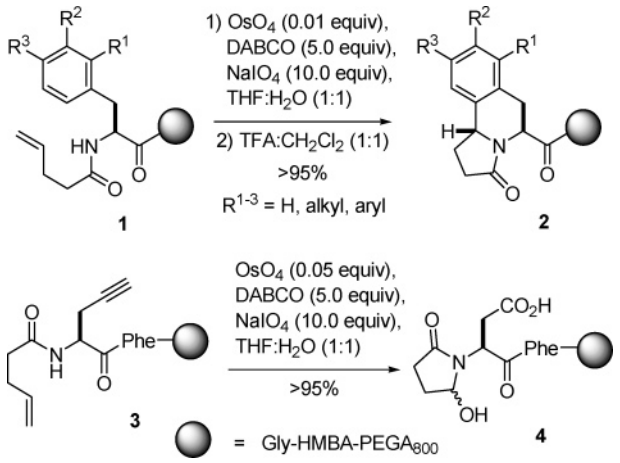
experiments was how the addition of DABCO allowed clean aldehyde generation, notably by excluding the formation of otherwise observed hydroxymethyl ketone side products.

A recent observation was that substrates containing alkyne functionalities (**3**) were converted to carboxylic acids (**4**) under similar sets of reaction conditions (Scheme 1). These

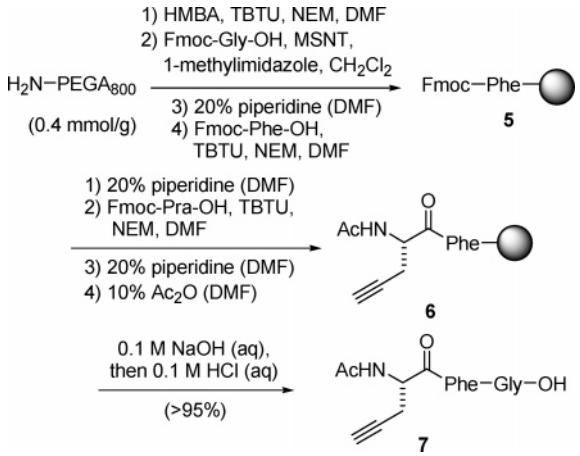
Scheme 1. OsO_4/$NaIO_4$/DABCO-Mediated Oxidative Cleavage of Solid-Supported Alkene and Alkyne-Containing Peptides



findings prompted us to further explore this route to solid-supported carboxylic acids in detail. Traditionally, the solution-phase oxidative cleavage of alkynes has been carried out with a variety of reagents, such as ozone,[9] alkaline hydrogen peroxide,[10] potassium permanganate,[11] and ruthenium tetroxide.[12] A number of reports have dealt with osmium tetroxide mediated variants,[13] but there have been no systematic investigations on the effect of added bases. We now wish to report how base additives enable quantitative solid-phase transformations of alkynes to the corresponding carboxylic and oxamic acids.

Acetylenic peptide **6** was synthesized in >95% purity (Scheme 2) and used as the initial test substrate for finding optimal reaction conditions for the desired transformation. The outcome of the OsO_4/$NaIO_4$-mediated oxidative cleavage reaction of alkyne **6** was found to depend strongly on the

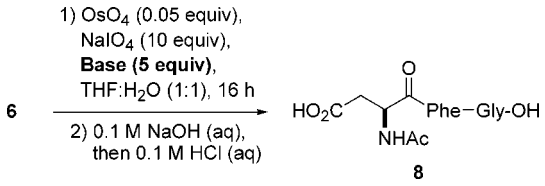
Scheme 2. Solid-Phase Synthesis of Acetylenic Peptide



base.[14] Extensive screening of bases revealed that the structurally related bases DABCO and HMTA were required additives for quantitative conversions (Table 1, entries 4 and 5).

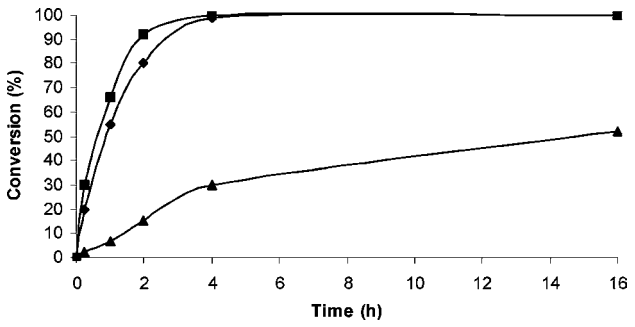
In further experiments, the compatibility of the reaction with various amino acid residues was tested (Table 2), as

Table 1. Screening of Bases in the OsO_4/$NaIO_4$-Mediated Oxidative Cleavage of Alkynes to Carboxylic Acids



entry	base	purity (%)[a]
1	no base	49
2	Et_3N	25
3	DBU	51[b]
4	DABCO	>95
5	HMTA	>95
6	2,6-lutidine	52
7	pyridine	60
8	DMAP	61

[a] In general, conversions were clean, thus providing a measure of the reaction purity (determined by RP-HPLC/MS). [b] Significant cleavage (>95%) of the linker was observed. Plot of the relative rates of oxidative cleavage reactions of alkyne (**6**) to the corresponding carboxylic acid mediated by OsO_4−$NaIO_4$ and selected bases: (◆) DABCO, (■) HMTA, and (▲) 2,6-lutidine:



(7) (a) Groth, T.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 34−44. (b) Groth, T.; Meldal, M. *J. Comb. Chem.* **2001**, *3*, 45−63. (c) Nielsen, T. E.; Meldal, M. *J. Org. Chem.* **2004**, *69*, 3765−3773. (d) Nielsen, T. E.; Meldal, M. *J. Comb. Chem.* **2005**, *7*, 599−610. (e) Nielsen, T. E.; Le Quement, S.; Meldal, M. *Org. Lett.* **2005**, *7*, 3601−3604. (f) Nielsen, T. E.; Le Quement, S.; Meldal, M. *Tetrahedron Lett.* **2005**, *46*, 7959−7962. (g) Diness, F.; Beyer, J.; Meldal, M. *Chem. Eur. J.* **2006**, *12*, 8056−8066.

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(9) Ando, W.; Miyazaki, H.; Ito, K.; Auchi, D. *Tetrahedron Lett.* **1982**, *23*, 555−556.

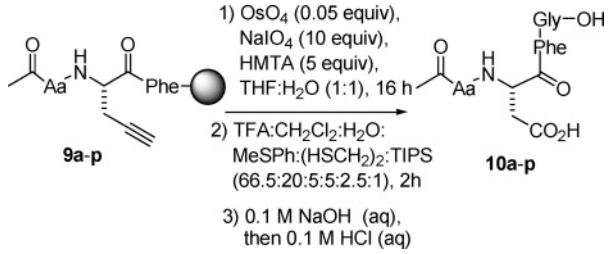
(10) Sawaki, Y.; Inoue, H.; Ogata, Y. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 1133−1138.

(11) Lee, D. G.; Chang, V. S. *J. Org. Chem.* **1979**, *44*, 2726−2730. Lai, S.; Lee, D. G. *Tetrahedron* **2002**, *58*, 9879−9887.

(12) Yang, D.; Chen, F.; Dong, Z.-M.; Zhang, D.-W. *J. Org. Chem.* **2004**, *69*, 2221−2223.

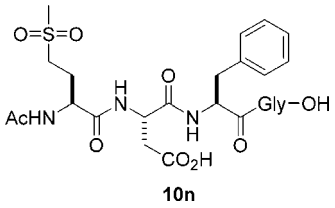
(13) For examples, see: (a) Jacobi, P. A.; Zheng, W. *Tetrahedron Lett.* **1993**, *34*, 2585−2588. (b) Czernecki, S.; Horns, S.; Valery, J.-M. *J. Org. Chem.* **1995**, *60*, 650−655. (c) Jacobi, P. A.; Murphree, S.; Rupprecht, F.; Zheng, W. *J. Org. Chem.* **1996**, *61*, 2413−2427.

Table 2. OsO_4/$NaIO_4$/HMTA-Mediated Oxidative Cleavage of Acetylenic Peptides to Carboxylic Acids



entry	amino acid residue (substrate)	product, purity (%)[a]
1	Ala (**9a**)	**10a**, >95
2	Val (**9b**)	**10b**, >95
3	Pro (**9c**)	**10c**, >95
4	Ile (**9d**)	**10d**, >95
5	L-Dap(Boc) (**9e**)[b]	**10e**, 85[c]
6	Trp (**9f**)	**10f**, 32
7	Ser(*t*-Bu) (**9g**)	**10g**, >95[c]
8	Ser(Bzl) (**9h**)	**10h**, >95
9	Thr (**9i**)	**10i**, >95
10	Gln (**9j**)	**10j**, >95
11	Gln(Trt) (**9j′**)	**10j**, >95[c]
12	Asp(O*t*-Bu) (**9k**)	**10k**, >95[c]
13	Arg(Pmc) (**9l**)	**10l**, >95[c]
14	Hyp(*t*-Bu) (**9m**)	**10m**, >95[c]
15	Met (**9n**)	**10n**, >95[d]
16	Tyr (**9o**)	**10o**, >95
17	Tyr(*t*-Bu) (**9o**)	**10o**, >95[c]
18	His(Trt) (**9p**)	**10p**, >95[d]

[a] The purity was determined by RP-HPLC/MS. [b] Dap (1,3-diaminopropionic acid). [c] All protecting groups of the functionalized amino acid side chains were fully removed by the acidic conditions indicated in step 2. [d] The sulfide moiety of the methionine-derived peptide was cleanly oxidized during the oxidation event to the corresponding sulfone **10n**.

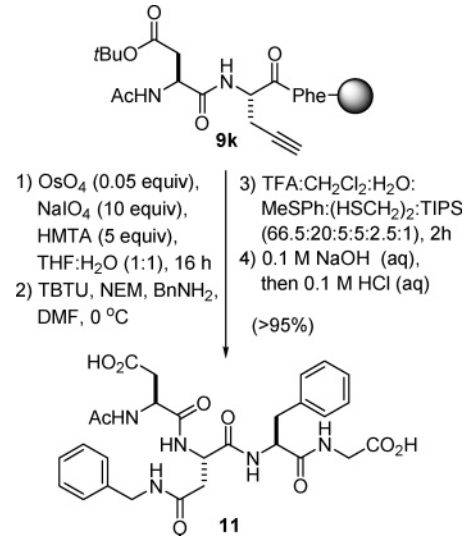


10n

illustrated for the synthesis of Asp-containing peptides **10a**–**p**. The high purity of the reaction products underlines the potential of using the acetylenic moiety as a masked carboxylic acid in the synthesis of aspartic acid derivatives, thus complementing the use of traditional protecting groups for the carboxylic acid functionality. To illustrate this point, substrate **9k** was treated with OsO_4/$NaIO_4$/HMTA to give an Asp(O*t*Bu)-Asp(OH)-containing intermediate, which upon TBTU activation/coupling with benzylamine, acid-mediated deprotection, and liberation from the solid support provided the selectively functionalized Asp(OH)-Asp(NHBn)-containing peptide **11** (Scheme 3) in excellent purity (>95%). The methodology is not limited to terminal alkynes, as illustrated for the synthesis of oxamic acid **13** (Scheme 4), which could

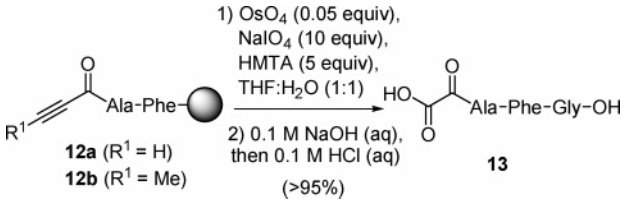
(14) Consult Supporting Information for an extensive table of bases screened.

Scheme 3. Solid-Phase Synthesis of Asp(OH)-Asp(NHBn)-Containing Peptide



be cleanly prepared from both the propynoic amide **12a** and 3-butynoic amide **12b**.

Scheme 4. OsO_4/$NaIO_4$/HMTA-Mediated Oxidative Cleavage of Propiolic Amides



The recent interest in utilizing oxamic acids (oxalic acid monoamides) as pharmacophores, e.g., as phosphate,[15] or pyruvate[16] mimics in the design of new enzyme inhibitors, prompted us to use the alkyne oxidation as a general strategy for incorporating this moiety in peptides. Rewardingly, the two most commonly used protecting groups in solid-phase peptide synthesis, Fmoc and Boc, proved fully compatible with the reaction conditions (Scheme 5). Bis-protected

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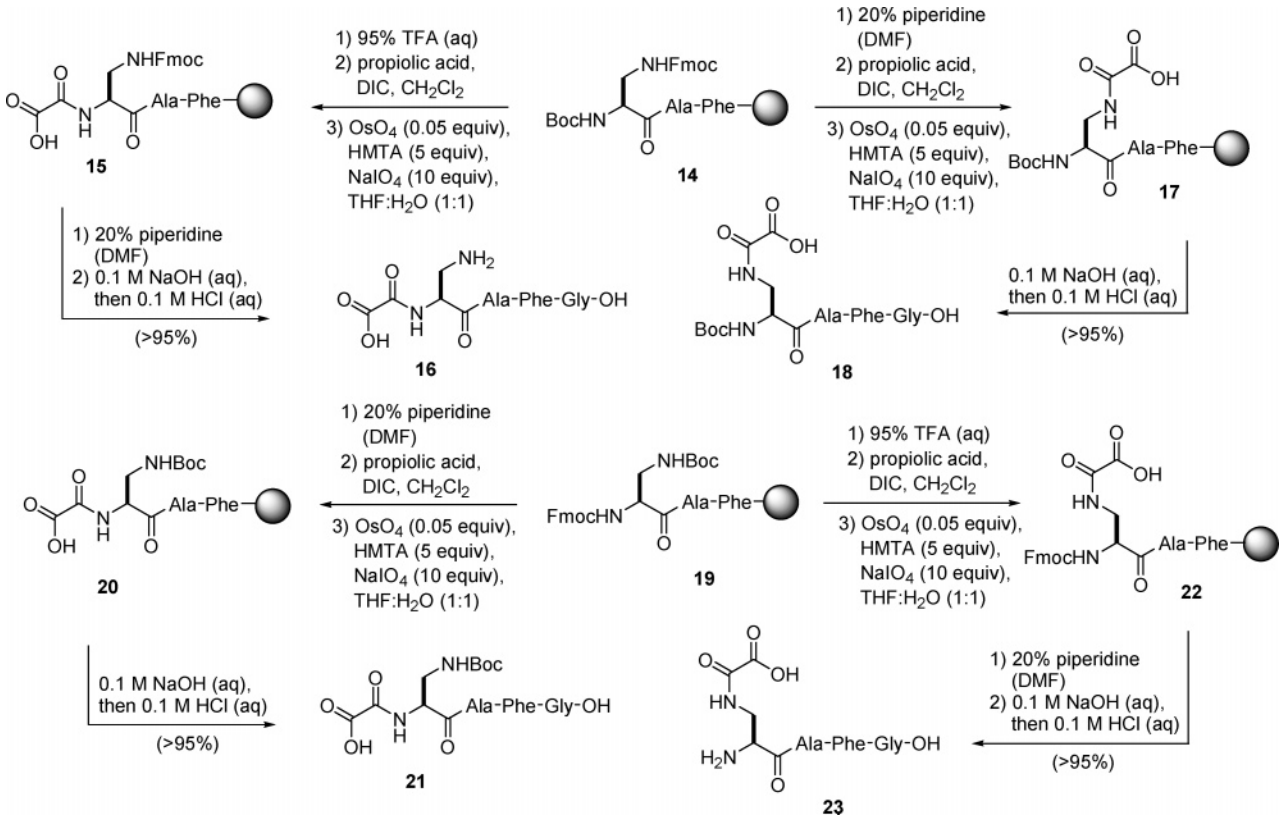
(17) **Representative Procedure for Oxidative Cleavage of Acetylenic Peptides.** A suspension of solid-supported alkyne **6** (1.0 equiv, 0,006 mmol, 20 mg), $NaIO_4$ (10.0 equiv, 0,06 mmol, 13 mg), and HMTA (5.0 equiv, 0,03 mmol, 4 mg) in THF/water (1:1) was shaken for 10 min, after which time OsO_4 (0.05 equiv, 0,3 μmol, 4 μL of a 2.5 wt % solution in 2-methyl-2-propanol) was added. The initially reddish reaction mixture was shaken for 16 h at 20 °C. Subsequently, the resin was washed with water (×6), 10% TFA (aq) (×3), water (×6), DMF (×6), and CH_2Cl_2 (×6). The resin was lyophilized to remove all traces of solvent. For release of material **7** from the solid phase, beads were treated with 0.1 M NaOH (aq) for 2 h, then neutralized with the equimolar amount of 0.1 M HCl (aq), and finally diluted with CH_3CN. The resulting solution was filtered and analyzed by RP-HPLC/MS.

Scheme 5. $OsO_4/NaIO_4$/HMTA-Mediated Oxidative Cleavage of Fmoc- and Boc-Protected Propiolic Amides



derivatives **14** and **19** were selectively transformed into the full matrix of monoprotected oxamic acids (**16**, **18**, **21**, and **23**), thus illustrating the applicability of the methodology with the standard protecting group scheme of solid-phase peptide synthesis.

In summary, we have developed a method for oxidizing solid-supported alkynes to the corresponding carboxylic and oxamic acids.[17] A key discovery was the addition of HMTA (or DABCO) to the reaction mixture, which ensures a clean, quantitative oxidative cleavage of the triple bond.

Acknowledgment. The Danish National Research Foundation and Carlsberg Laboratory are gratefully acknowledged for financial support.

Supporting Information Available: Analytical data (HPLC, MS and NMR) for all compounds cleaved from the solid support. This material is available free of charge via the Internet at http://pubs.acs.org.

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