ORGANIC LETTERS

2006
Vol. 8, No. 25
5817−5820

Carbocations in Action. Design, Synthesis, and Evaluation of a Highly Acid-Sensitive Naphthalene-Based Backbone Amide Linker for Solid-Phase Synthesis

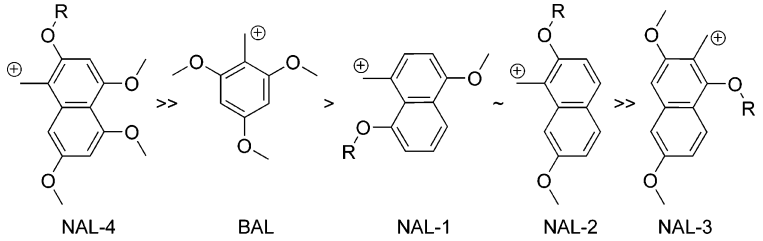
Michael Pittelkow, Ulrik Boas, and Jørn B. Christensen*

Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100, Copenhagen Ø, Denmark

jbc@kiku.dk

Received September 30, 2006

ABSTRACT



The design, synthesis, and properties of an extremely acid-labile backbone amide linker based on a regiospecifically substituted tetraalkoxy naphthaldehyde core are presented. This handle enables cleavage of peptide backbone amides (secondary amides) off a solid support using as little as 0.5% TFA in CH_2Cl_2. This proceeds without cleavage of *tert*-butyl ethers and *tert*-butyl esters. The design is based on a DFT study that predicted the most stabile alkoxy-substituted methyl naphthyl carbocation.

Acid-labile handles remain the most widely used in solid-phase organic synthesis, and there is an immense interest in handles with high acid lability enabling release of the substrate from the solid phase under mild conditions. To this end, one important issue is the possibility of performing controlled release of protected peptide segments for further manipulation by segment coupling strategies.[1−3]

The Barany and Ellman groups introduced the backbone amide linker (BAL) concept, which enables release of an amide-containing substrate, e.g., peptides to be released in a "traceless" manner by using a backbone amide functionality as the anchor point to the solid phase (Scheme 1).[4,5] The acid lability of a BAL-type handle correlates to a large extent with the ease of formation of the benzyl-like carbocation, which forms during release of the product from the resin (for leaving groups with comparable electronic and steric properties).[6] Solid-phase synthesis utilizing a BAL strategy starts at an aromatic aldehyde situated on an electron-rich aromatic core. In solid-phase synthesis of peptides by the BAL strategy, the first step is a reductive amination of the BAL formyl group with the amino group of a C-terminal

(1) (a) Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149. (b) Letsinger, R. L.; Kornet, M. J. *J. Am. Chem. Soc.* **1963**, *85*, 3045.

(2) (a) *Methods of Organic Chemistry*; Goodman, M., Felix, A., Moroder, L., Toniolo, C., Eds.; Houben-Weyl, Georg Thieme Verlag: Stuttgart, New York, 2002; Vol. E22a. (b) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (c) Merrifield, R. B. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 799.

(3) For general reviews on handles: (a) Songster, M. F.; Barany, G. *Methods Enzymol.* **1997**, *289*, 126. (b) Backes, B. J.; Ellman, J. A. *Curr. Opin. Chem. Biol.* **1997**, *1*, 86. (c) James, I. W. *Tetrahedron* **1999**, *55*, 4855. (d) Virta, P.; Katajisto, J.; Niittyma, T.; Lönnberg, H. *Tetrahedron* **2003**, *59*, 5137.

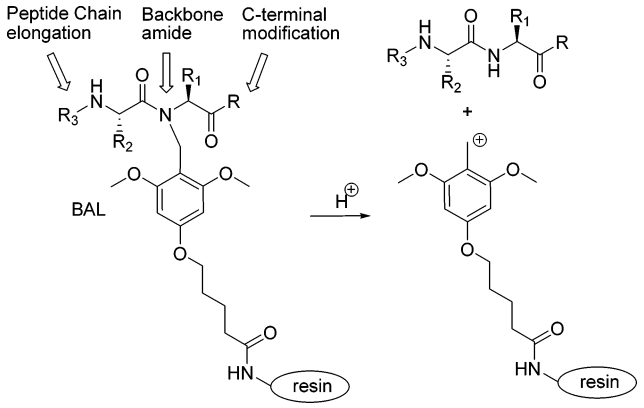
(4) Jensen, K. J.; Songster, M. F.; Vágner, J.; Alsina, J.; Albericio, F.; Barany, G. *J. Am. Chem. Soc.* **1998**, *120*, 5441.

(5) (a) Boojamra, C. G.; Burow, K. M.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 5742. (b) Boojamra, C. G.; Burow, K. M.; Thompson, L. A.; Ellman, J. A. *J. Org. Chem.* **1997**, *62*, 1240.

(6) Other factors may influence the cleavage properties: Norrby, P.-O.; Jensen, K. J. *Int. J. Pept. Res. Ther.* **2006**, in press.

10.1021/ol062410j CCC: $33.50
Published on Web 11/10/2006

Scheme 1. Cleavages of a Secondary Amide (Peptide Backbone Amide) off a BAL-Type Handle Releasing a Stabilized Benzylic Carbocation



protected amino acid.[7] The secondary amine product is acylated with an N-protected amino acid to create the backbone amide linkage (BAL linkage). This opens the possibility of modifying both the C-terminal and the N-terminal ends of a given peptide or other amide-containing substrates. This was first demonstrated with a 2,4,6-trialkoxy benzene moiety as the aromatic core of the handle and has since been studied applying a variety of different aromatic and heteroaromatic core structures (Scheme 1).[8,9]

Following the synthesis and evaluation of three different methoxy-substituted NAL handles (naphthalene backbone amide linker), a number of factors proved important for achieving maximum carbocation stabilization using extended polycyclic aromatic hydrocarbons.[10,11] The carbocations involved in the cleavage from the previously described handles (NAL-1, NAL-2, and NAL-3) and the newly designed handle (NAL-4) are shown in Figure 1 indicating

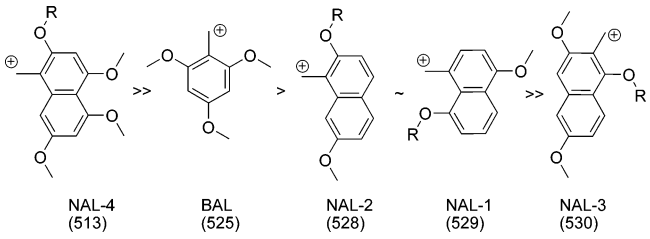
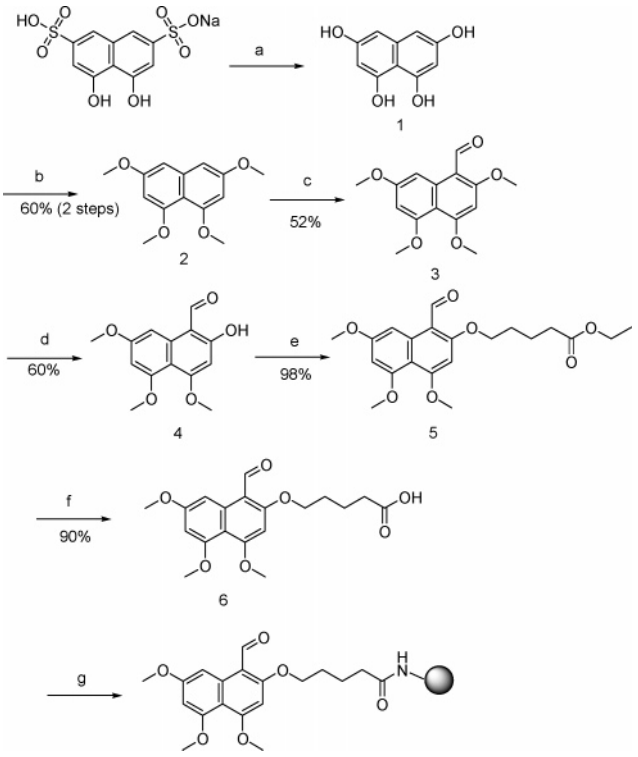


Figure 1. Carbocations according to the handle they are formed from. The relative ease of formation of carbocations is indicated. R indicates the point of attachment to the solid support. Values in brackets refer to the heat of formation from the arene in kilocalories/mole.[13]

the relative ease of formation. Especially, we were intrigued by the observation that the trialkoxy naphthalene-based handle (NAL-3, Figure 1) was less acid labile than the two dialkoxy-based handles (NAL-1 and NAL-2, Figure 1), and all of these three proved less acid labile than the trialkoxy benzene derivative originally described.[10−12] To understand these results, we used DFT calculations to predict the energy requirements for the formation of various methoxy-substituted methyl naphthyl carbocations.[13] These calculations showed that the energy requirements for the formation of the carbocation are sensitive to a number of factors such as substitution pattern, planarity of the aromatic core, and the *peri*-effect. The most pronounced destabilizing effect proved to be overcrowding of the periphery of the aromatic core which forces the substituents out of the aromatic plane, thus interrupting the conjugation. These computational considerations pointed in the direction of a 2,4,5,7-tetramethoxy-1-naphthyl methyl carbocationic species as the most stable methoxy-substituted naphthyl methyl carbocation (NAL-4, Figure 1).[13]

In continuation of our work toward more acid-labile NAL-type handles, we present the synthesis and properties of a handle (NAL-4) based on 2,4,5,7-tetramethoxy-1-naphthaldehyde (Scheme 2).

Scheme 2. Synthesis of NALdehyde-4 (**6**)[a]



[a] Reagents and conditions: (a) $Ba(OH)_2 \times 8H_2O$, KOH, NaOH, 250 °C, 12 h; then (b) dimethylsulfate, acetone, K_2CO_3, 48 h, 60% (two steps); (c) $POCl_3$, DMF, −5 °C, 52%; (d) BBr_3, CH_2Cl_2, 60%; (e) 5-bromovaleric acid ethyl ester, DMF, K_2CO_3, 98%; (f) NaOH, THF, 90%; (g) PyBOP, DMF, DIEA.

The synthetic sequence starts by alkali melting of chromotropic acid using $Ba(OH)_2 \cdot 8H_2O$, KOH, and NaOH followed by methylation of the crude 1,3,6,8-tetrahydroxynaphthalene (**1**) with dimethylsulfate in anhydrous acetone to give 1,3,6,8-tetramethoxynaphthalene (**2**) in 60% yield.[14,15]

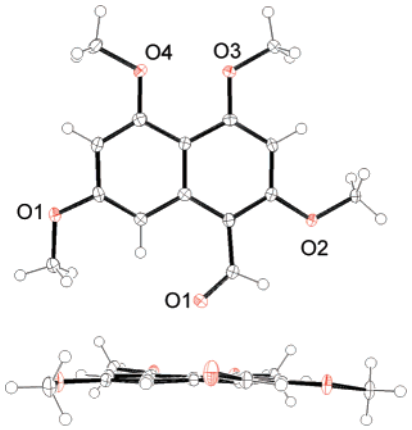


Figure 2. X-ray crystal structure of compound **3**. In this structure, all carbon and oxygen substituents are roughly in the plane of the naphthalene core.

Vilsmeier−Haack formylation ($POCl_3$ in DMF) in dilute solution at −5 °C regiospecifically introduced the aldehyde in the α-position in moderate yield (52%). Demethylation with BBr_3 in refluxing CH_2Cl_2 was ortho selective relative to the aldehyde and gave the naphthol (**4**) in 60% isolated yield. Alkylation of the naphthol with 5-bromovaleric acid ethyl ester in DMF with K_2CO_3 gave the ester (**5**) in 98% yield, and hydrolysis in THF/NaOH gave NALdehyde-4 (**6**) in 90% yield.

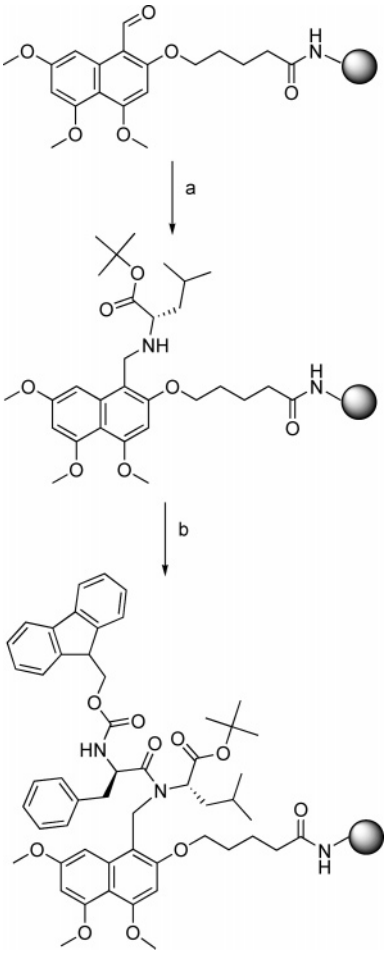
Table 1. Cleavage Yields of Fmoc−Phe-Ala−OH Using Different Conditions

cleavage conditions[a]	Fmoc−Phe-Ala−OH yield (%)[b]	Fmoc−Phe-Ala−O^{t}Bu yield (%)[b]
TFA−H_2O 95:5	61	0
TFA−DCM 1:1	90	0
TFA−DCM 5:95	37	62
TFA−TIS−DCM 5:5:90	33	62
TFA−DCM 1:99	0	68
TFA−TIS−DCM 1:5:94	0	71
TFA−DCM 5:995	0	63
TFA−DCM 1:999	0	9

[a] All cleavage experiments were conducted at room temperature for 2 h. [b] Cleavage yields were calculated by comparison with a standard curve.

The single-crystal X-ray structure of **3** (Figure 2 and Supporting Information) confirms the regiochemistry of the synthetic procedure (vide infra). In addition, it shows the crucial planarity of the aromatic core and the alkoxy substituents. DFT calculations have shown that this planarity has a large impact on the stability of the extended benzylic carbocation.[13]

Scheme 3. Anchoring of H−Leu−O^{t}Bu and Fmoc−Phe−OH to the NAL-4 Derivatized Solid Phase[a]

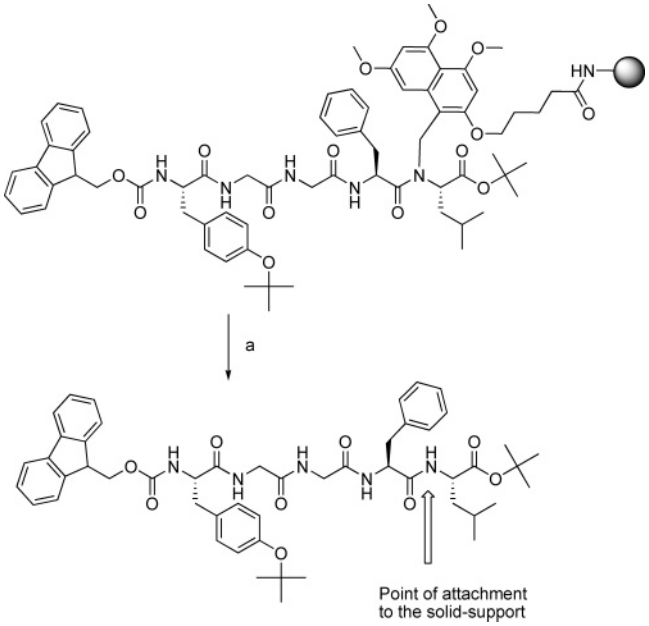


[a] Reagents and conditions: (a) H−Leu−O^{t}Bu×HCl, $NaBH_3CN$, DMF; (b) Fmoc−Phe−OH, DMF, DIPCDI.

Subsequently, the NAL-4 handle (**6**) was anchored to a high-loading amino-methylated polystyrene resin by PyBOP-mediated amide bond formation and a model dipeptide was synthesized (Fmoc−Phe-Ala−O^{t}Bu).[7a] The synthesized dipeptide was cleaved from the solid support using different acidic conditions (Table 1). These cleavage experiments showed that the fully protected dipeptide was cleaved off in high yield using as low as 0.5% TFA in CH_2Cl_2. This result is unprecedented in BAL-type solid-phase synthesis. Cleaving

(7) For reviews of BAL handles, see: (a) Alsina, J.; Jensen, K. J.; Albericio, F.; Barany, G. *Chem.−Eur. J.* **1999**, *5*, 2787. (b) Alsina, J.; Jensen, K. J.; Songster, M. F.; Vágner, J.; Albericio, F.; Barany, G. In *Solid-phase Organic Synthesis*; Czarnik, A. W., Ed.; John Wiley & Sons: New York, 2001; Vol. 1, p 121.
(8) For an indole-based BAL handle, see: Estep, K. G.; Neipp, C. E.; Stramiello-Stephens, L. M.; Adam, M. D.; Allen, M. P.; Robinson, S.; Roskamp, E. J. *J. Org. Chem.* **1998**, *63*, 5300.
(9) Jessing, M.; Brandt, M.; Jensen, K. J.; Christensen, J. B.; Boas, U. *J. Org. Chem.* **2006**, *71*, 6734.
(10) Boas, U.; Christensen, J. B.; Jensen, K. J. *J. Comb. Chem.* **2004**, *6*, 497.
(11) Pittelkow, M.; Boas, U.; Jessing, M.; Jensen, K. J.; Christensen, J. B. *Org. Biomol. Chem.* **2005**, *3*, 508.
(12) Boas, U.; Brask, J.; Christensen, J. B.; Jensen, K. J. *J. Comb. Chem.* **2002**, *4*, 223.
(13) Pittelkow, M.; Christensen, J. B.; Sølling, T. I. *Org. Biomol. Chem.* **2005**, *3*, 2441.
(14) Our procedure for the synthesis of **1** is similar to: Tanaka, H.; Ohne, Y.; Ogawa, N; Tamura, T. *Agric. Biol. Chem.* **1963**, *27*, 48.
(15) For a recent and different strategy to **2**: McCulloch, M. W. B.; Barrow, R. A. *Tetrahedron Lett.* **2005**, *46*, 7619.

Scheme 4. Cleavage of Fmoc−Tyr(O^{t}Bu)−Gly-Gly-Phe-Leu−O^{t}Bu from the NAL-4 Derivatized Solid Phase[a]



[a] Reagents and conditions: (a) 0.5% TFA/1% TIS/CH_2Cl_2.

the dipeptide off under stronger acidic conditions provided the peptide with a C-terminal acid in yields that are comparable with the yields obtained with the original BAL handle.

Finally, the pentapeptide Fmoc−Tyr(O^{t}Bu)−Gly-Gly-Phe-Leu−O^{t}Bu was synthesized on the NAL-4 functionalized solid support by solid-phase synthesis. This peptide has a Fmoc, a *tert*-butyl ether, and a *tert*-butyl ester as protecting groups. The anchoring of the first two amino acids is shown in Scheme 3. The first amino acid was attached via a reductive amination using H−Leu−O^{t}Bu·HCl as the amine, and the second amino acid was added using Fmoc−Phe−OH and DIPCDI as the coupling reagent.

The remaining portion of the pentapeptide was synthesized using a standard solid-phase synthesis protocol.[7] The pentapeptide was cleaved off without cleavage of any of the three protecting groups (Scheme 4).

In conclusion, we have designed, synthesized, and demonstrated the high acid lability of a regiospecifically designed tetraalkoxy-substituted NAL handle. The design was based on DFT calculations of a series of model compounds, and this technique provides a powerful tool in the design of materials based on extended aromatic structures.

NAL-4 is the most acid-labile handle for BAL-type solid-phase synthesis presented to date, and it makes cleavage of secondary amide substrates with 0.5% TFA in CH_2Cl_2 possible.

Acknowledgment. We gratefully acknowledge Flemming Hansen for collecting the crystal structure data and the Center for Crystallographic Studies for the use of their equipment. Associate Professor Theis I. Sølling (University of Copenhagen) and Associate Professor Knud J. Jensen (KVL) are acknowledged for fruitful discussions throughout the course of this project.

Supporting Information Available: Full experimental procedures and characterization of all compounds are provided. CIF file for compound **3** is also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

OL062410J