ORGANIC LETTERS

2011
Vol. 13, No. 15
3770–3773

Versatile Method for the Detection of Covalently Bound Substrates on Solid Supports by DART Mass Spectrometry

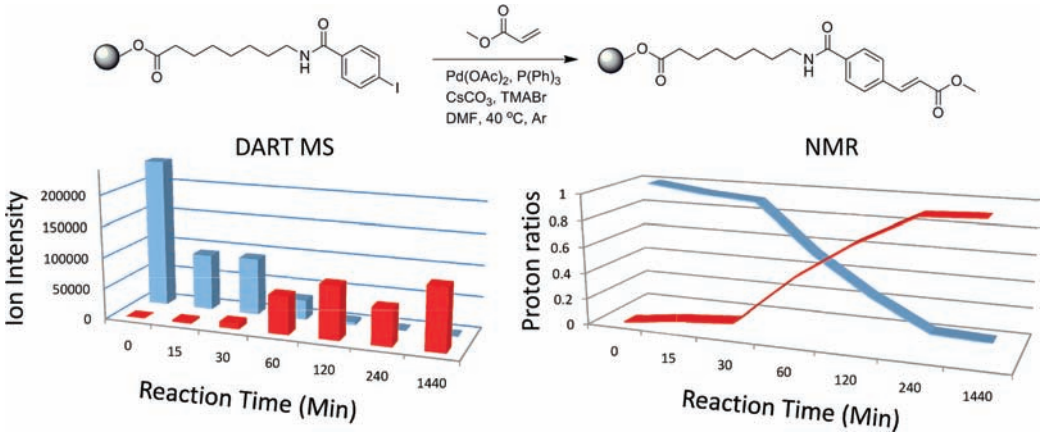
Laura M. Sanchez,[†] Matthew E. Curtis,[‡] Bianca E. Bracamonte,[†] Kenji L. Kurita,[†] Gabriel Navarro,[†] O. David Sparkman,[‡] and Roger G. Linington*,[†]

Department of Chemistry and Biochemistry, University of California Santa Cruz, Santa Cruz, California 95064, United States, and Pacific Mass Spectrometry Facility, Department of Chemistry, College of the Pacific, University of the Pacific, Stockton, California 95211, United States

rogerl@chemistry.ucsc.edu

Received March 7, 2011

ABSTRACT



Analysis of substrates directly on solid phase resins without the need for separate cleavage conditions remains an outstanding challenge in the field of solid phase synthesis. We now present the first example of simultaneous cleavage and mass spectrometric analysis of peptides from solid supports using direct analysis in real time (DART) mass spectrometry. We have shown that this method is compatible with a diverse array of solid phase resins and is suitable for analysis of both peptides and organic substrates.

Solid phase peptide synthesis (SPPS) is now a mature field, with hundreds of applications, ranging from the creation of split-pool libraries,[1] to the commercial preparation of vaccines[2] and clinical therapeutics.[3] As an extension of this technology, solid phase organic synthesis (SPOS) has developed into a mature field over the past decade and is now a mainstream technique for the creation of small molecule libraries.[4] Despite the widespread adoption of solid phase synthesis technology, the direct analysis of resin-bound substrates remains an elusive goal. Current analytical methods include FTIR,[5] LC-MS,[6] magic angle spinning NMR,[7] and colorimetric tests.[6] Generally, these methods require cleavage of the substrate from the resin, precluding further chemical manipulation. These methods

[†] University of California Santa Cruz.
[‡] University of the Pacific.

(1) Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84–86.
(2) Arnon, R.; Tarrab-Hazdai, R.; Steward, M. *Immunology* **2000**, *101*, 555–562.
(3) Verlander, M. *Int. J. Pept. Res. Ther.* **2007**, *13*, 75–82.
(4) Moura-Letts, G.; DiBlasi, C. M.; Bauer, R. A.; Tan, D. S. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6745–6750.
(5) (a) Yan, B.; Gremlich, H. U. *J. Chromatogr. B* **1999**, *725*, 91–102. (b) Yan, B.; Yan, H. *J. Comb. Chem.* **2001**, *3*, 78–84.
(6) Scicinski, J. J.; Congreve, M. S.; Kay, C.; Ley, S. V. *Curr. Med. Chem.* **2002**, *9*, 2103–2127.
(7) (a) Arai, T.; Fujiwara, A.; Watanabe, M.; Yokoyama, N.; Fujito, T.; Deguchi, K.; Yanagisawa, A. *Tetrahedron Lett.* **2006**, *47*, 1673–1677. (b) Rousselot-Pailley, P.; Ede, N. J.; Lippens, G. *J. Comb. Chem.* **2001**, *3*, 559–563.

10.1021/ol201404v
Published on Web 06/24/2011

are low throughput and typically require the sacrifice of the cleaved material, thus reducing overall yields.

Previously, researchers have gone to great lengths to install mixed linkers[8] or to employ highly technical instrumentation (e.g., nanomanipulation and various MALDI techniques)[9] to extract information about substrates without requiring global cleavage from the resin. In particular, time-of-flight-secondary ion mass spectrometry (TOF-SIMS) has been shown to be effective for ionization of surface associated peptides from polystyrene beads. This method can be coupled with the release of peptides from solid supports with TFA vapors, but failed to yield analyte ions when extended to covalently bound substrates.[10] Photocleavable linkers that are activated by short pulses from MALDI lasers have also been used to simultaneously remove and analyze substrates from resin based solid supports.[11] In addition, there are several recent reports that employ electron impact mass spectrometry to the analysis of polystyrene resins bearing simple surface functionalizations.[12] We now present the first example of the use of direct analysis in real time mass spectrometry (DART-MS) for the analysis of resin bound substrates and demonstrate that this technique is appropriate for a diverse array of SPPS and SPOS products. The method can be adapted to determine peptide sequence from MS fragmentation studies and can also be used to monitor reaction progress in SPOS systems.

The field of mass spectrometry is currently undergoing a period of intense technological advancement, centered on the design and development of new ionization methods. Among these new techniques, desorption electrospray ionization (DESI) and DART have emerged as two new approaches for the analysis of heterogeneous substrates under atmospheric pressure conditions. Together with advances in TOF-SIMS[13] and MALDI, these techniques now offer the ability to analyze a diverse array of heterogeneous substrates, from mass spectral imaging of cryosectioned biological samples,[14] to the detection of explosives residue on clothing.[15]

In DART analysis, ionization is achieved through the collision of metastable excited-state neutral helium atoms,

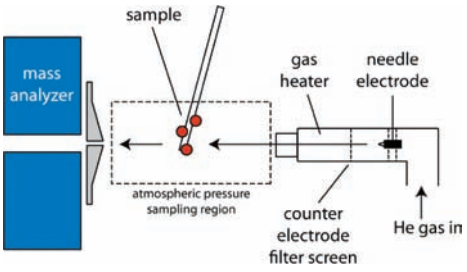


Figure 1. Schematic of DART ionization source.

formed by atmospheric pressure glow discharge, with water molecules within the atmospheric pressure region of the ionization source, to form protonated water dimers (Figure 1). Subsequent proton transfer from these water dimers to analyte molecules affords analyte ions that can be detected using standard mass analyzer technologies.[16] The principle advantages of DART ionization over other techniques are the high degree of tolerance for sample heterogeneity, and elimination of requirement for sample preparation. This provides an opportunity for the investigation of materials that have previously been inaccessible to mass spectrometric analysis. Given the recent rash of reports detailing heterogeneous substrate analysis using DART, we were motivated to explore the potential for using this technology to directly analyze resin-bound peptides without prior chemical cleavage.

Our initial study was performed with a small (15 μM) solid phase synthesis lantern, containing a peptide scaffold of relevance to our global health drug discovery program.[17] The washed lantern (3× DMF, 3× CH_2Cl_2, 3× DMF) was exposed to the ionization region of the source, and gave immediate strong signals for both $[M - Fmoc + H]^+$ and various y ions (Supporting Information, Figure S12), showing that it was possible to readily observe predicted protonated signals for covalently attached analytes using DART-MS. Encouraged by this initial success, we expanded the scope of the study to include both the most commonly used polymer supports (SPPS resins, lanterns, and TentaGel), and the most frequently used linkers (trityl, Rink amide, Wang, and Merrifield). The same substrate (Fmoc-VVVAF-resin) was synthesized on each of the solid supports using standard SPPS methods (Supporting Information), and analyzed by DART-MS under identical conditions. In total we synthesized 12 substrates for analysis, containing different combinations of solid supports and linkers. These included four polystyrene-based resin analogues, three TentaGel samples, and two lantern analogues. In all cases we were able to observe either $[M + H]^+$ or $[M - Fmoc + H]^+$ ions, indicating that cleavage and ionization of analytes is independent of both polymer support and linker composition.

(8) Townsend, J. B.; Shaheen, F.; Liu, R.; Lam, K. S. *J. Comb. Chem.* **2010**, *12*, 700–712.

(9) (a) Brown, J. M.; Hoffmann, W. D.; Alvey, C. M.; Wood, A. R.; Verbeck, G. F.; Petros, R. A. *Anal. Biochem.* **2010**, *398*, 7–14. (b) Greving, M. P.; Kumar, P.; Zhao, Z. G.; Woodbury, N. W. *Langmuir* **2010**, *26*, 1456–1459.

(10) Brummel, C. L.; Lee, I. N.; Zhou, Y.; Benkovic, S. J.; Winograd, N. *Science* **1994**, *264*, 399–402.

(11) (a) Gerdes, J. M.; Waldmann, H. *J. Comb. Chem.* **2003**, *5*, 814–820. (b) Semmler, A.; Weber, R.; Przybylski, M.; Wittmann, V. *J. Am. Soc. Mass. Spectrom.* **2009**, *21*, 215–219.

(12) (a) Heinze, K.; Winterhalter, U.; Jannack, T. *Chem.—Eur. J.* **2000**, *6*, 4203–4210. (b) Chavez, D.; Ochoa, A.; Madrigal, D.; Castillo, M.; Espinoza, K.; Gonzalez, T.; Velez, E.; Melendez, J.; Garcia, J. D.; Rivero, I. A. *J. Comb. Chem.* **2003**, *5*, 149–154.

(13) (a) Kim, Y. P.; Oh, E.; Shon, H. K.; Moon, D. W.; Lee, T. G.; Kim, H. S. *Appl. Surf. Sci.* **2008**, *255*, 1064–1067. (b) Min, H.; Kim, Y.; Yu, H.; Moon, D. W.; Lim, S. J.; Yoon, H. J.; Lee, T. G.; Shin, S. K. *Chem.—Eur. J.* **2008**, *14*, 8461–8464.

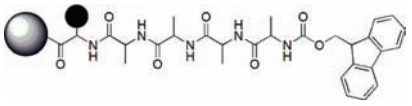
(14) Esquenazi, E.; Yang, Y. L.; Watrous, J.; Gerwick, W. H.; Dorrestein, P. C. *Nat. Prod. Rep.* **2009**, *26*, 1521–1534.

(15) Cody, R. B.; Laramee, J. A.; Durst, H. D. *Anal. Chem.* **2005**, *77*, 2297–2302.

(16) Cody, R. B. *Anal. Chem.* **2009**, *81*, 1101–1107.

(17) Sanchez, L. M.; Lopez, D.; Vesely, B. A.; Della Togna, G.; Gerwick, W. H.; Kyle, D. E.; Linington, R. G. *J. Med. Chem.* **2010**, *53*, 4187–4197.

Table 1. Amino Acid Compatibility[a]



polar	acidic	basic	aliphatic	aromatic
Ser(*t*-Bu)	Asp(O-*t*-Bu)	Lys(Boc)	Gly	Phe
Thr(*t*-Bu)	Glu(O-*t*-Bu)	$Arg(Boc)_2$[b]	Ala	Tyr(*t*-Bu)
Cys(*t*-Bu)[c]		His(Boc)[c]	Val	Trp(Boc)
Pro			Leu	
Asn(Dmcp)[c]			$Met(O_2)$	
Gln(Dmcp)[c]			Ile	

[a] Grey sphere = resin support; black sphere = variable residue. [b] When the Pfb protecting group was used, no y ions were observed. [c] When the Tr group was used, only $[Tr]^+$ ions were observed.

To be of general utility, any analytical method for SPPS analytes needs to show substrate tolerance for each of the 20 proteinogenic amino acids. A library of 20 pentapeptides was therefore synthesized, with the general structure Fmoc-AAAAX-resin, where X was replaced with each amino acid in turn (Table 1). Analysis of these samples afforded $[M - Fmoc + H]^+$ and y ions in all cases, showing that this method is suitable for analytes containing any of the standard amino acids.

Although identification of parent masses is a valuable tool for SPPS chemistry, the determination of complete amino acid sequences directly from solid phase resins would significantly increase the utility of this method for investigating samples such as split-pool synthesis libraries. Given that MS/MS fragmentation is a widely used method for investigating amino acid sequence in proteomics research, we elected to examine the effect of applying CAD fragmentation to solid phase substrates for the generation of fragmentation spectra for peptide sequencing. Examination of 2-chlorotrityl-bound Fmoc-VVVAF-resin using in-source collisionally activated dissociation (CAD) afforded a near-universal fragmentation pattern, including all predicted a, b, and y ions (Figure 2). This high coverage of fragmentation patterns is uncommon in peptide mass spectrometry. However, using this DART-CAD method, we were able to unequivocally assign the sequence of the test substrate directly from fragmentation data.

In addition to the use of SPPS in modern biotechnology research, SPOS, employing organic substrates, is also a valuable tool for library development. To examine the suitability of our new method for nonpeptidic substrates, we prepared a library of organic small molecules for analysis using this technique. A total of 18 organic acids (Figure 3) were coupled to 2-chlorotrityl resin and separately analyzed using standard DART-MS conditions. All 18 of these substrates, including basic, polar, aliphatic and aromatic substrates, afforded ions consistent with predicted cleavage products, indicating that this method is widely applicable to both SPPS and SPOS substrates.

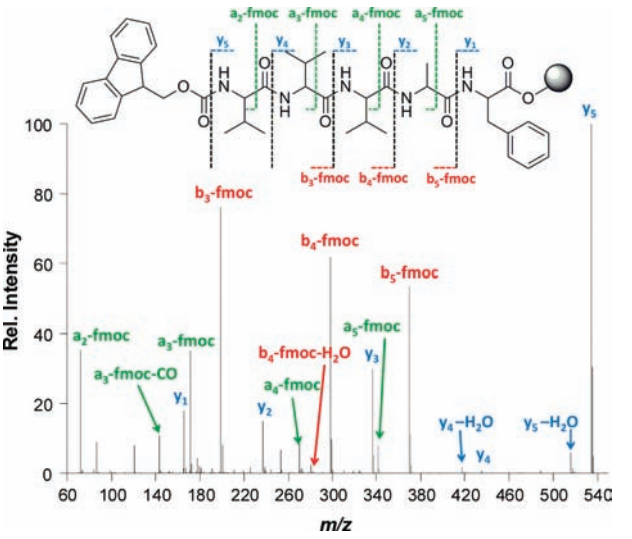


Figure 2. CAD fragmentation spectrum.

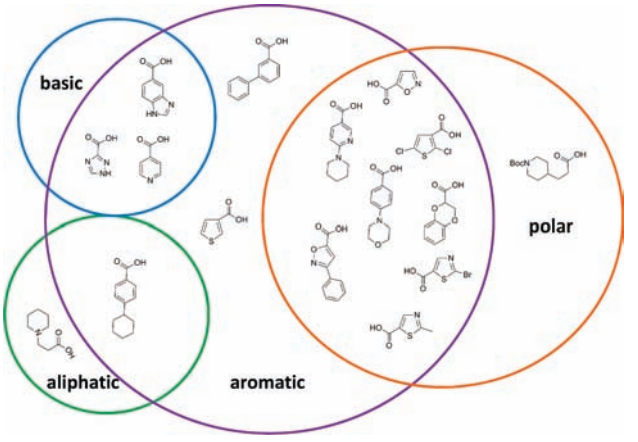


Figure 3. Library of SPOS substrates, grouped by functionality.

Finally, we explored the potential for the use of this method for following reaction progress on the solid support. Reaction monitoring of SPOS reactions is notoriously challenging, with most current methods requiring quenching, cleavage and HPLC analysis of aliquots of resin to check reaction progress. This strategy is time-consuming, and precludes real-time monitoring of reaction progress because of the inherent delay caused by the HPLC step. We were therefore motivated to explore the application of this new analytical tool to on-bead monitoring of reaction progress.

An on-bead Heck coupling between aryl iodide (**1**) and methyl acrylate (**2**) was selected as a representative example of the type of complex synthetic transformation being performed in modern SPOS chemistry. The reaction was followed by removing an aliquot of beads at each time

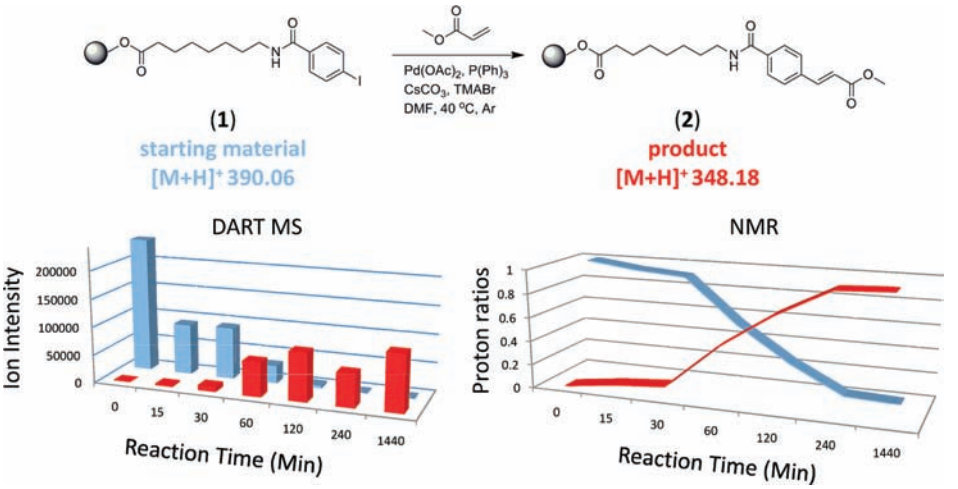


Figure 4. On-bead Heck reaction followed by DART-MS and verified with ^{1}H NMR.

point, and washing them thoroughly with 10% $NaCO_3$ followed by 3× DMF, 3× CH_2Cl_2, 3× DMF. Analysis of the beads by DART-MS showed a time-dependent decrease in ion intensity for ions consistent with the molecular weight of the starting material, coupled with a corresponding increase in intensity for ions consistent with the predicted reaction product (Figure 4). A parallel examination of the starting material/product ratio by cleavage and NMR analysis of the same beads showed comparable results, indicating that DART-MS can be used for the qualitative monitoring of reaction progress. It is important to stress that this reaction monitoring method is not quantitative, because of the combined effects that differential ionization, ion suppression and variation in sample concentrations within the ion source can have on apparent ion intensities. As a mass-based analytical technique, this method is best suited to the monitoring of starting material and product ions, and is not necessarily suitable for identifying side reactions, whose masses are unknown. Nevertheless, this method offers a new approach to the qualitative evaluation of reaction progress on solid supports.

In conclusion, we have presented the first example of the analysis of covalently attached analytes on solid surfaces by DART-MS. This new method is applicable to the analysis of peptides, and can be used to determine peptide sequence directly on the solid support using in-source CAD fragmentation. The method has been shown to work with a variety of acid-labile linkers, and all of the standard amino acids, including amino acids containing side chain protecting groups. It should be noted that protecting groups are often lost during the ionization process and that ions such as $[M - Fmoc + H]^+$ are common; in addition, both Pfb and Tr protecting groups should be avoided as they cause signal interference that precludes the observation of analyte ions. Finally, we have shown that this method is applicable to synthetic organic substrates such as aromatic heterocycles and can be used for qualitative real-time reaction monitoring without sample preparation.

Acknowledgment. We thank Aaptec, Varian, and Rapp PolymereGmBH for the donation of solid supports, and P.R. Jones for helpful discussions. Financial support was provided by the University of California Santa Cruz startup funds, the International Cooperative Biodiversity Group (ICBG) program in Panama (Grant ICBG TW006634), the NIH NIGMS Bridges to the future program (GM 51765-10) and NSF graduate research fellowships (L.M.S. and G.N.).

Supporting Information Available. DART-MS spectra for all tested resins, experimental procedures for solid phase synthesis, DART-MS analysis, and plot of carrier gas temperature dependence on ion signal strength. This material is available free of charge via the Internet at http://pubs.acs.org.