Analytical Techniques for Combinatorial Chemistry: Quantitative Infrared Spectroscopic Measurements of Deuterium-Labeled Protecting Groups

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Abstract: Infrared microspectroscopy has been developed as a powerful, nondestructive technique for the quantitative analysis of solid-phase, resin-bound chemical reactions. The synthesis and/or application of deuterium isotope containing protecting groups, including acetyl-d_3 chloride, benzoyl-d_5 chloride, and 2-[[[(*tert*-butyl-d_9)oxy]carbonyl]-oximino]-2-phenylacetonitrile (BOC-ON-d_9) are also introduced as highly selective infrared signatures amenable to quantitation by infrared spectroscopy. Calculation of first derivative carbon−deuterium (C−D) stretching absorbance relative to resin backbone derivative absorbances provided pathlength-independent quantitation of deuterium content and allowed for the determination of chemical yields in solid-phase resin reactions involving changes in deuterium content.

Introduction

The advent of combinatorial methods has led to a renewed interest in solid-phase-based chemistry. While the advantages of solid-phase synthesis have been best illustrated in the generation of peptides and oligonucleotides,[1,2] other chemistries have been less well explored. A major impediment to the development of new synthetic methodology is the absence of fast, nondestructive analytical methods. TLC, HPLC, GC, and MS techniques are clearly not feasible unless the compound of interest is first cleaved from the polymeric support. The requirement for cleavage of the compound from the resin for analysis renders the method inherently destructive. Of greater concern is the uncertainty involved in the cleavage chemistry. As the structure of the resin bound molecule changes in the course of a synthesis, so does the rate of cleavage, such that a high-yielding reaction may be misinterpreted as failing due to a poor cleavage reaction. It is possible to reduce the sensitivity of the cleavage rate to the structure of the bound molecule by separating the two with a chemical spacer. It is also possible to increase the reactivity of the linker to cleavage. However, the synthetic approach often does not allow for the use of such "extenders", and reactive linkers can lead to premature cleavage during the course of the synthesis.

A more flexible and expedient method of following the course of reactions on solid-phase supports is direct analysis of the compound on the resin. Gel phase NMR methods (both ^{1}H and ^{13}C) show promise, especially when using magic angle spinning techniques.[3,4] However, sensitivity limitations (particularly when low resin loadings are used), spectral interference by the signals resulting from the resin backbone, and difficulties in obtaining accurate integrals, combined with broad lines, hinder spectral interpretation. Infrared microspectroscopy provides a sensitive nondestructive means of monitoring resin-based reactions directly on the solid-phase support.[5] Direct functional group analysis provided by infrared spectroscopy permits qualitative analysis of each reaction step throughout the synthesis.[6] However, spectral overlap for similar functional groups in complex molecules, coupled with variability of the cross-sectional molar absorbance, often limits the usefulness of infrared spectroscopy for qualitative and, in particular, quantitative analysis.

Results and Discussion

Substitution of a reactant with its deuterium-labeled counterpart has proven to greatly enhance the functional group selectivity of infrared analysis by enabling the resultant carbon−deuterium (C−D) stretching absorbance to be observed in a largely interference-free spectral region from 2300 to 2200 cm^{-1}. Figure 1 illustrates the infrared spectrum collected from the amide coupling product obtained by reaction of aminomethyl polystyrene resin with benzoyl-d_5 chloride. The aromatic C−D stretching absorbance at 2275 cm^{-1} is clearly resolved from C−H absorbances in the 3100−2700 cm^{-1} spectral region in Figure 1. In this spectrum the amide NH (3400−3200 cm^{-1}) and C═O (1670 cm^{-1}) absorbances are also observed against the styrene background.

In order to simulate benzoylation reaction products of different yet known yields, needed for quantitation of the C−D stretching absorbance in this reaction, mixtures of benzoyl-d_5

† Lead Discovery Department.

⊗ Abstract published in *Advance ACS Abstracts,* August 15, 1996.

(1) Houghten R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature* **1991**, *354*, 84−86.

(2) Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmierski, W. M.; Knapp, R. J. *Nature* **1991**, *354*, 82−84.

(3) Chen, J. K.; Schreiber, S. L. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 953−969.

(4) Anderson, R. C.; Jarema, M. A.; Shapiro, M. J.; Stokes, J. P.; Ziliox, M. *J. Org. Chem.* **1995**, *60*, 2650−2651.

(5) Spectra are collected by one of two methods depending on pathlength/sensitivity and bead size of the particular analysis. Short pathlength spectra required for strongly absorbing function groups are obtained using infrared microscopy in conjunction with a diamond anvil compression cell. Higher sensitivity spectra required for weakly absorbing or low concentration functional groups are obtained from a single bead imbedded in a KBr disk. The KBr disk not only serves as a support substrate for transmission analysis of the bead but also reduces spectral artifacts and lensing of the bead by matching the index of refraction of the bead to the parallel surfaces of the KBr disk. Nonlinearity is further minimized by analysis of a small cross section of the bead diameter.

(6) Yan, B.; Kumaravel, G.; Anjaria, H.; Wu, A.; Petter, R. C.; Jewell, C. F., Jr.; Wareing, J. R. *J. Org. Chem.* **1995**, *60*, 5736−5738.

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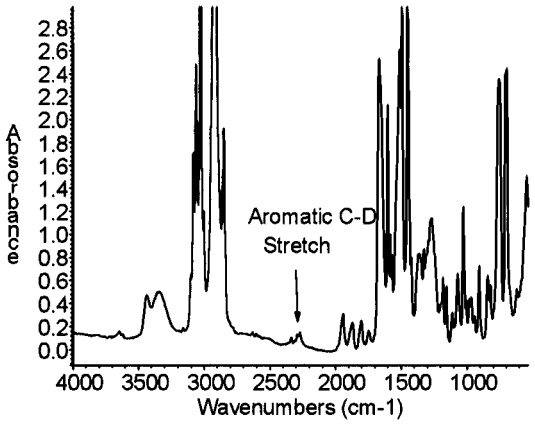
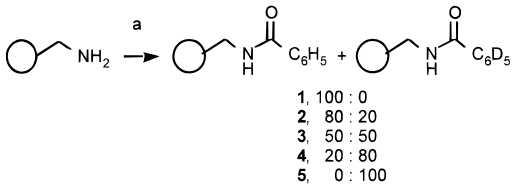


Figure 1. Infrared spectrum of a deuterium-labeled amide coupling product.

Scheme 1[a]



[a] Reagents: (a) triethylamine, 4-(dimethylamino)pyridine, benzoyl chloride/benzoyl-d_5 chloride (100:0, 80:20, 50:50, 20:80, 0:100).

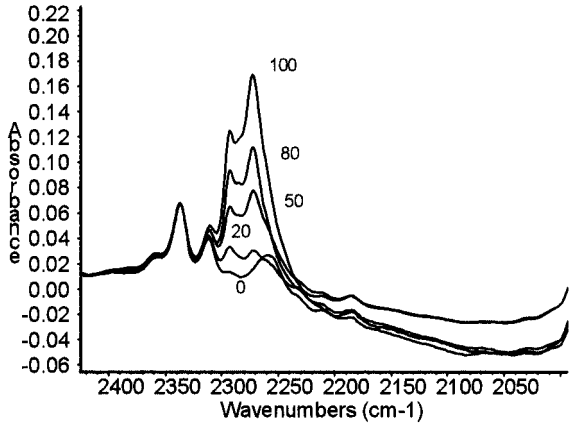


Figure 2. C−D stretch region for 0% (**1**), 20% (**2**), 50% (**3**), 80% (**4**), and 100% (**5**) deuteration.

chloride and benzoyl chloride were prepared and reacted with (aminomethyl)polystyrene resin as outlined in Scheme 1. In this instance, both acid chlorides should react at the same rate since the isotope is far removed from the reaction center. Complete reaction of the (aminomethyl)polystyrene resin was insured by the fact that subsequent treatment with excess reagent for prolonged reaction times had no effect on the overall reaction yield.

Figure 2 illustrates the C−D spectral absorbance region for the series of amides generated in the Scheme 1 reactions. Good visual correlation between relative deuterium loading and the C−D stretching absorbance was apparent. However, baseline variability present in the spectra make quantitative baseline selection very subjective; thus data in the linear absorbance format is of limited quantitative utility, especially in the case of low reaction yields.

Calculation of first-derivative spectra, with subsequent Savitsky−Golay smoothing, greatly simplified baseline selection, as illustrated in Figure 3. Using first-derivative data, a classical least squares (K-Matrix) calibration was performed using the 2290.60 cm^{-1} derivative band height without baseline correction.

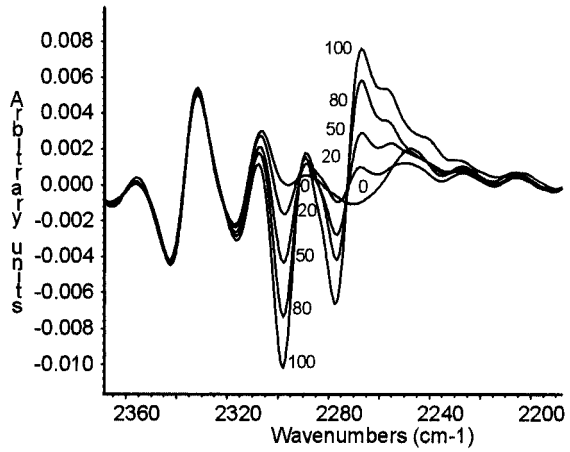


Figure 3. C−D derivative spectra for 0% (**1**), 20% (**2**), 50% (**3**), 80% (**4**), and 100% (**5**) deuteration.

Table 1. Amide Coupling Statistical Data

sample	theoretical	calculated	difference
1	0.0000	0.5434	0.5434
2	20.0000	19.1451	−0.8549
3	50.0000	48.8319	−1.1681
4	80.0000	81.0521	1.0521
5	100.0000	100.1459	0.1459
		average difference = 0.94	

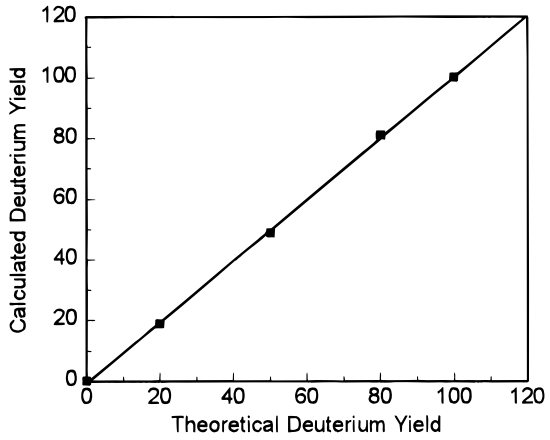


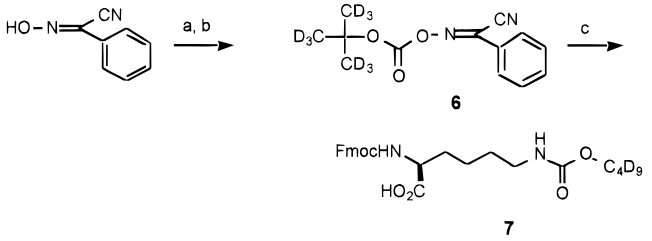
Figure 4. Calculated vs theoretical amide coupling results.

In this sample set, the 2356.4−2342.9 cm^{-1} polystyrene derivative absorbance difference was used as an internal pathlength reference against which all analytical derivative bands were normalized. This internal standardization procedure was used to compensate for bead curvature and inconsistent bead to bead diameters, which result in large pathlength variations. Theoretical vs calculated data for the C−D stretching calibration are listed in Table 1 and illustrated in Figure 4. For these data, theoretical values refer to the synthetic ratios used for generation of the concentration calibration. Calculated values were obtained by subsequently treating each standard as an unknown and calculating its value using the theoretical value regression.

To further the scope of these analyses, calibration experiments were also conducted for perdeuteriobenzoylation of alcohols and perdeuterioacetylation of alcohols and amines. In each case, excellent correlation between the relative deuterium loading and the C−D stretching absorbance was obtained, reinforcing the quantitative potential of this technique. However, discrete calibrations were found to be necessary for each type of coupling, i.e. amide or ester.

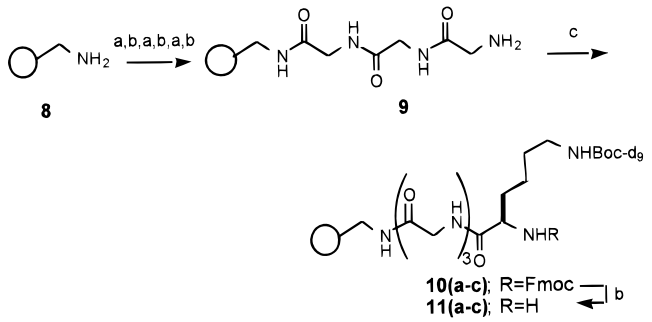
Analogous technology has also been extended to the analysis of deuterated side chain protecting groups for amino acids. For

Scheme 2[a]



[a] Reagents: (a) triphosgene, *N*,*N*-dimethylaniline; (b) $(CD_3)_3COD$, pyridine; (c) Fmoc-Lys-OH, Et_3N, dioxane, water.

Scheme 3[a]



[a] Reagents: (a) Fmoc-Gly-OH, 1,3-diisopropylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF; (b) 20% piperidine/DMF; (c) **7**, 1,3-diisopropylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF.

peptide synthesis techniques using the *N*-(fluorenylmethoxycarbonyl) (Fmoc) protecting group, side chains are usually protected with acid labile groups, i.e. *tert*-butyl esters, or ethers and *tert*-butyloxycarbonyl (Boc) protected amines. Incorporation of deuterium into these protecting groups provides a method for monitoring the coupling reactions as the peptide chain is being elongated. Removal of the labeled protecting groups during the standard deprotection step, i.e. TFA cocktail, permits isolation of the desired peptide, without "memory" of the deuterated protection. Thus, the deprotection step can also be monitored by C−D infrared absorbance.

2-[[[(*tert*-Butyl-d_9)oxy]carbonyl]oximino]-2-phenylacetonitrile (BOC-ON-d_9, **6**) was prepared by reaction of 2-(hydroxyimino)-2-phenylacetonitrile with triphosgene followed by trapping with 2-methyl-2-propanol-d_{10} as shown in Scheme 2.[7] Further reaction with α-*N*-(fluorenylmethoxycarbonyl)-L-lysine [Fmoc-Lys-OH] in the presence of base gave the diprotected amino acid Fmoc-Lys-(Boc-d_9)-OH (**7**).[8]

In Scheme 3, aminomethyl resin **8**[9,10] was coupled to three glycine residues to provide a spacer between the terminal amino residue and the polystyrene resin, thus allowing for more reproducible coupling reactions. The resulting amino terminal resin **9** was then split into three batches and each coupled with **7** to give the Fmoc-protected terminal amines **10a**−**c**. Deprotection of **10a**−**c** gave **11a**−**c**. These six resin samples, each containing one Boc-d_9 group, were used as the standards for the Boc-d_9 infrared C−D absorbance.

Table 2 presents calculated vs theoretical data obtained from calibration of the 2222.4 cm^{-1} derivative intensity for samples **10a**−**c**, **11a**−**c**, and the nondeuterio analog **12** (Scheme 4). In this calibration, analytical absorbance bands were normalized to the 2608.1−2596.6 cm^{-1} polystyrene internal reference band to compensate for pathlength differences. It should be noted that the 2596.6 internal standard was inaccessible due to baseline slope in the linear absorbance spectrum mode; however, derivative analysis provided excellent calibration with an average difference of 2.14%. Spectra acquisition times of greater than 1 min could be implemented to further reduce scatter in the calibration resulting from signal to noise limitations of the derivative spectra. Bead inhomogeneity, however, can only be addressed by the analysis of multiple beads.

Table 2. Boc-d_9 Calibration Results

sample	theoretical	calculated	% difference
10a	1.00	1.0257	2.57
10b	1.00	0.9603	−3.97
10c	1.00	1.0062	0.62
11a	1.00	0.9763	−2.37
11b	1.00	1.0196	1.96
11c	1.00	0.9868	−1.32
		average difference = 2.14%	

Table 3. Boc-d_9 Quantitation Results

sample	theoretical	calculated	% difference
13	1.00	1.0104	1.04
14	1.00	1.0932	9.32
15	1.00	1.0005	0.05
17	1.00	1.1045	10.45
18	1.00	1.1423	14.23
		average difference = 7.02%	

The utility of this infrared technique would be limited if calibrations needed to be performed for every reaction in question. However, if the Boc-d_9 C−D absorbance was independent of the Boc-d_9 environment, then a single calibration could be used for a broad range of reactions. To explore this possibility, compounds **13**, **14**, **15**, **17**, and **18** in which the Boc-d_9 group is present in different chemical environments were prepared as shown in Scheme 4. Samples **13** and **14** contain Fmoc-protected and unprotected lysine separated by a lysine residue from the Gly-Gly-Gly spacer. Sample **15** results from direct coupling of Boc-d_9 to the spacer. Finally, samples **17** and **18** contain Fmoc-protected and unprotected lysine separated from the spacer by a Lys-Glu dipeptide.

Table 3 lists theoretical vs calculated results for compounds **13**, **14**, **15**, **17**, and **18** using the calibration data generated for samples **10a**−**c** and **11a**−**c**. From the Table 3 data, it is apparent that matrix effects imposed by the different chemical environment of each sample had negligible effect on Boc-d_9 quantitation by infrared absorbance, thus permitting universal calibration of this protecting group regardless of matrix, within the scope of these experiments.

As a further illustration of the power of this technique, samples containing multiple Boc-d_9-protected lysines were prepared according to Scheme 5. Samples **19**−**24** were then quantified using an extrapolation of the Boc-d_9 calibration generated with samples **10a**−**c** and **11a**−**c**. Theoretical vs calculated data are presented in Figure 5 and Table 4. An excellent correlation between the number of Boc-d_9 lysines and derivative infrared absorbance was obtained. Again, it should be noted that the calibration is based on an extrapolation of the regression generated in Table 1 and does not result from internal regression of samples **19**−**24**. The technique is thus capable of "counting" Boc-d_9-protected lysines.

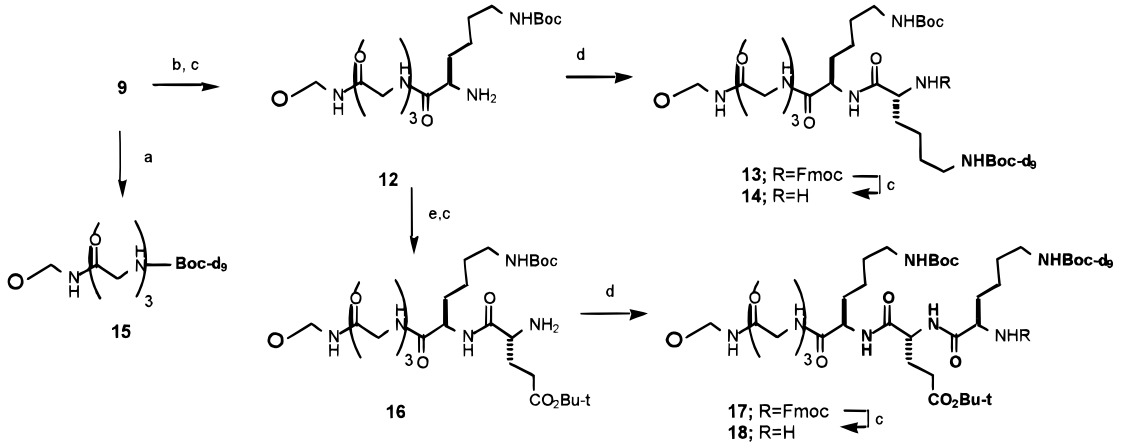
(7) Itoh, M.; Hagiwara, D.; Kamiya, T. A new *tert*-butyloxycarbonylating reagent, 2-*tert*-butyloxycarbonyloxyimino-2-phenylacetonitrile. *Tetrahedron Lett*. **1975**, *49*, 4393−4394.

(8) Paleveda, W. J.; Holly, F. W.; Veber, D. F. *Organic Synthesis*; Wiley: New York, 1990; Collect. Vol. VII, pp 75−77.

(9) Polymer Laboratories PL-CMS Resin 1.0 mequiv/g.

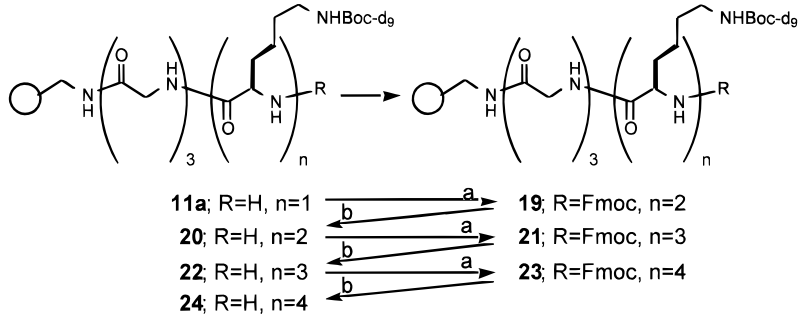
(10) Weinshenker, N. M.; Shen, C. M.; Wong, J. Y. *Org. Synth*. **1977**, *56*, 95−99.

Scheme 4[a]



[a] Reagents: (a) **7**, Et_3N; (b) Fmoc-Lys(Boc)-OH, 1,3-diisopropylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF; (c) 20% piperidine/DMF; (d) Fmoc-Lys(Boc-d_9)-OH, 1,3-diisopropylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF; (e) Fmoc-Glu(OBu-t)-OH, 1,3-diisopropylcarbodiimide, 1-hydroxybenzotriazole hydrate, DMF.

Scheme 5[a]



[a] Reagents: (a) **7**, 1,3-diisopropylcabodiimde, 1-hydroxybenzotriazole hydrate, DMF; (b) 20% piperidine/DMF.

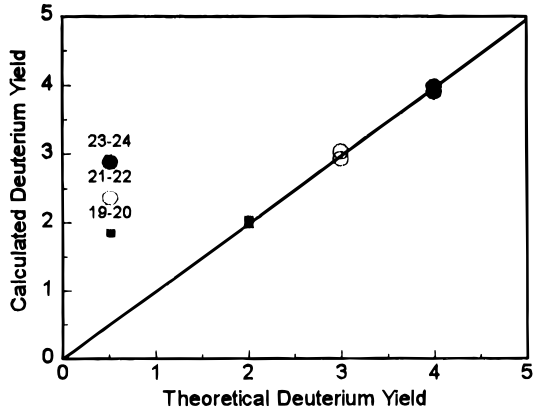


Figure 5. Calculated vs theoretical D_9 content using the Table 2 calibration standards.

Table 4. Extrapolated Boc-d_9 Quantitation Results

sample	theoretical	calculated	% difference
19	2.00	1.9723	−1.34
20	2.00	2.0205	1.03
21	3.00	2.9199	−2.67
22	3.00	3.0244	0.81
23	4.00	3.9032	−2.42
24	4.00	3.9888	0.28
		average difference = 1.43%	

Experimental Section

All reagents were obtained from Aldrich Chemical Co. Inc. (Milwaukee, WI).

Standard Procedure for Coupling Amino Acids to (Aminomethyl)polystyrene Resin. (Aminomethyl)polystyrene resin[9,10] was placed in a bond elute tube and washed with DMF (5×). A minimum amount of DMF was added so that bubbling N_2 gas caused the mixture to mix efficiently. Fmoc-Gly-OH (3 equiv), 1-hydroxybenzotriazole hydrate (HOBt) (5 equiv), and 1,3-diisopropylcarbodiimide (DIC) (3.5 equiv) were added. N_2 bubbling was continued for 2 h then the resin was filtered and washed with DMF (5×). A small sample was removed for testing by the Kaiser test.[11] If the test was positive the coupling was repeated.

Standard Procedure for Fmoc Group Removal. The resin was washed with DMF (5×). A 20% solution of piperidine in DMF was added with N_2 bubbling for 5 min. The resin was filtered and washed with DMF (5×). The deprotection was repeated for 10 min and then the resin was rewashed with DMF (5×). A sample was removed and tested to ensure a positive Kaiser test.

Standard Procedure for IR Sampling. The resin was washed with CH_2Cl_2 (5×) and then dried by drawing air through the resin for 10−15 min. Sample beads were then imbedded in KBr and pressed into a pellet for analysis by direct transmission infrared microscopy. In each case, the infrared beam was apertured to a single bead in the KBr pellet. Spectrometer parameters are as follows: Nicolet Magna System 750 (Nicolet Instruments, Madison, WI), MCT/A detector KBr beamsplitter, 100 scans (54.3 s), 4 cm^{-1} resolution, one level of zero filling, Happ−Genzel apodization.

Infrared Quantitation. All peak height, regression, and quantitation calculations were performed using Nicolet's "Omnic Quant IR" data analysis software package. K-Matrix regression was implemented in each case.

[(Benzoylamino)methyl]polystyrene (1−5). Aminomethyl resin was placed in five bond elute tubes and washed with CH_2Cl_2 (5×). The resin was suspended in a minimum amount of CH_2Cl_2 with N_2

(11) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Analyst Biochem.* **1970**, *84*, 595−598.

bubbling, and triethylamine (5.05 equiv) and 4-(dimethylamino)pyridine (catalytic amount) were added to each tube. Mixtures of benzoyl-d_5 chloride and benzoyl chloride (100:0, 80:20, 50:50, 20:80, and 0:100) (5.05 equiv) were added. After 2 h the resin was drained and washed with DMF (5×), CH_2Cl_2 (5×), and methanol (5×).

2-[[[(*tert*-Butyl-d_9)oxy]carbonyl]oximino]-2-phenylacetonitrile (Boc-ON-d_9), 6. To a solution of 2-(hydroxyimino)-2-phenylacetonitrile (1.58 g, 10.8 mmol) and *N*,*N*-dimethylaniline (1.3 g) in dioxane (0.5 mL) and toluene (20 mL) at 0 °C was added a solution of triphosgene (1.07 g, 3.6 mmol). The resulting solution was stirred at room temperature for 16 h and then cooled to 0 °C. After a mixture of 2-methyl-2-propanol-d_{10} (1 mL) and pyridine (1.1 mL) in toluene (5 mL) had been added, the cooling bath was removed and the mixture stirred at 35 °C for 3 h. The reaction was diluted with CH_2Cl_2 (100 mL), washed with water (20 mL), dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography over silica gel (2.5 × 18 cm) with elution with a 5−10% ethyl acetate/hexane gradient. The fractions containing the product (R_f 0.54; 10% ethyl acetate/hexane) were combined and concentrated. The residue was crystallized from methanol/water (1:9) to give BOC-ON-d_9 (0.7 g, 25%) as pale yellow powder; mp 86.6 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46−7.60 (m, 3 H), 7.93−7.97 (m, 2 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 26.53 (septet), 85.42, 108.34, 127.32, 127.73, 129.15, 132.82, 137.45, 149.97. Anal. ($C_{13}H_5D_9N_2O_3$) C, H, N.

α-*N*-(Fluorenylmethoxycarbonyl)-ϵ-*N*-[[(*tert*-butyl-d_9)oxy]carbonyl]-L-lysine [Fmoc-Lys-(Boc-d_9)-OH], 7. BOC-ON-d_9 (0.3 g, 1.17 mmol) was added to a mixture of α-*N*-(fluorenylmethoxycarbonyl)-L-lysine [Fmoc-Lys-OH] (0.57 g, 1.4 mmol) and triethylamine (0.8 mL, 5.7 mmol) in water (10 mL) and dioxane (10 mL). The mixture was stirred at room temperature for 3 h, diluted with water (100 mL), and extracted with ether (2 × 20 mL) and ethyl acetate (2 × 20 mL). The aqueous phase was acidified with 1 N hydrochloric acid and extracted with CH_2Cl_2 (3 × 20 mL). All the organic extracts were combined, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography over silica gel (2.5 × 18 cm) with a 2−10% methanol/$CHCl_3$ gradient. The fractions containing the product (R_f 0.33; 10% methanol/$CHCl_3$) were combined and concentrated to give **7** (0.45 g, 80%) as a white powder; mp 65−67 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.3−1.96 (m, 6 H), 2.93−3.18 (m, 2 H), 4.13−4.81 (m, 5 H), 5.73−5.92 and 6.16−6.40 (m, 1 H), 7.19−7.79 (m 8 H), 7.79−8.50 (br s, 1 H); MS API+ 469 ($M^+ + 1$). Anal. ($C_{26}H_{23}D_9N_2O_6 \cdot 0.5H_2O$) C, H, N.

PS-Gly-Gly-Gly-NH_2 (9). Aminomethyl resin **8** was coupled with Fmoc-Gly-OH and the Fmoc group removed according to the standard procedures. These two step procedures were repeated three times to give **9**.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-NHFmoc (10a−c). Compound **9** was split into three different bond elute tubes and each coupled with Fmoc-Lys(Boc-d_9)-OH using the standard procedure to give **10a−c**.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-NH_2 (11a−c). Compounds **10a−c** were treated with piperidine using the general procedure to give **11a−c**.

PS-Gly-Gly-Gly-Lys(Boc)-NH_2 (12) was prepared from **9** by coupling with Fmoc-Lys(Boc)-OH and Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc)-Lys(Boc-d_9)-NHFmoc (13) was prepared from **12** by coupling with **7** under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc)-Lys(Boc-d_9)-NH_2 (14) was prepared from **13** by Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-NH-Boc-d_9 (15). Compound **9** was placed in a bond elute tube and washed with DMF (5×). A minimum amount of DMF was added along with BOC-ON-d_9 (2 equiv) and triethylamine (2 equiv). N_2 bubbling was continued for 8 h, then the resin was filtered and washed with DMF (5×).

PS-Gly-Gly-Gly-Lys(Boc)-Glu(OBu-t)-NH_2 (16) was prepared from **12** by coupling with Fmoc-Glu(OBu-t)-OH and Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc)-Glu(OBu-t)-Lys(Boc-d_9)-NHFmoc (17) was prepared from **16** by coupling with **7** and under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc)-Glu(OBu-t)-Lys(Boc-d_9)-NH_2 (18) was prepared from **17** by Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-NHFmoc (19) was prepared from **11a** by coupling with **7** and under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-NH_2 (20) was prepared from **19** by Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-NHFmoc (21) was prepared from **20** by coupling with **7** and under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-NH_2 (22) was prepared from **21** by Fmoc removal under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-NHFmoc (23) was prepared from **22** by coupling with **7** and under the standard procedures described above.

PS-Gly-Gly-Gly-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-Lys(Boc-d_9)-NH_2 (24) was prepared from **23** by Fmoc removal under the standard procedures described above.

Conclusions

Application of infrared microspectroscopy for the quantitative analysis of the C−D content in a solid-phase reagent has been demonstrated. This technique facilitates analysis of solid-phase reactions where the number of C−D bonds changes throughout the course of a reaction. The use of deuterated protecting groups such as Boc-d_9 provides for a simple method of labeling amino-containing substrates. The magnitude of the infrared absorbance from Boc-d_9 was found to be independent of environment, thus the number of lysines in a multiple lysine solid-phase sample could be determined. This technique should have broad utility in solid-phase combinatorial chemistry.

Supporting Information Available: Microanalysis data for deuterated compounds **6** and **7** (1 page). See any current masthead page for ordering and Internet access instructions.

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