Infrared and Raman Spectra of a Single Resin Bead for Analysis of Solid-Phase Reactions and Use in Encoding Combinatorial Libraries

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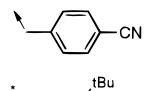
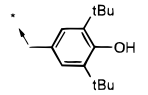
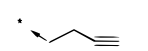
A range of lysine amides **2** to **7** tagged with different combinations of either Raman- and/or infrared (IR)-active groups (4-cyanobenzoyl chloride, 3,5-di-*tert*-butyl-4-hydroxybenzoic acid, or 4-pentynoic acid) have been synthesized on Sasrin resin. Randomly selected beads of unknown identity were then analyzed by Fourier transform infrared (FTIR) and Raman microspectrometry. Using a combination of these two spectroscopic techniques, the six amide derivatives **2** to **7** and the unfunctionalized lysine template **1** were unambiguously identified from their Raman and FTIR spectra. The potential applications of FTIR and Raman microspectrometry for analysis of organic reactions on solid support and for encoding combinatorial libraries has been demonstrated.

A combinatorial compound library is a collection of compounds constructed through a series of synthetic reactions, often on solid supports, with multiple reagent choices for each step.[1,2] The result is a chemical library with extensive molecular variation. The chemical identity of each discrete compound can be elucidated by a variety of methods, including attachment of tag molecules[3−5] to a resin bead, direct mass spectrometry,[6,7] or using colored and fluorescent beads.[8,9]

The discovery in our laboratories and by other groups,[10] that organic molecules attached to individual polystyrene beads can be identified using Fourier transform infrared (FTIR) microspectrometry, prompted us to investigate the use of this technique and also, for the first time, raman microspectrometry, in monitoring reactions on single resin beads. We report here a novel single-bead analysis method using a combination of Raman and FTIR microspectrometry. We also describe a novel approach to encoding combinatorial libraries based on IR and Raman spectroscopy. Our proposed strategy requires distinct IR-active and/or Raman-active tags to label individual components of the chemical library on an appropriate bridged scaffold using the cosynthesis method.[3] Alternatively, the libraries could be self-coded by incorporating IR and Raman active components.

Table 1. Frequency (cm^{-1}) of Infrared and Raman Tags

Tag	Structure	Infrared signal	Raman signal
R^1		2231	2230-2131
R^2		3624	N/A
R^3		2120	2119-2120

To test this potential encoding approach and to investigate a new analytical application of Raman microspectrometry, a library of polymer-bound lysine derivatives was prepared (see structure). Orthogonally protected lysine **1** attached to glycine-derivatized Sasrin resin was acylated with groups containing functionalities that have diagnostic IR or Raman absorptions. In this instance, to ensure unambiguous assignment of the IR and Raman spectra, only functional groups (Table 1) that have characteristic vibrational frequencies in the less crowded 4000−3500 cm^{-1} and 2800−1800 cm^{-1} regions were considered. The synthetic route is as follows: Fmoc-lysine-4,4-dimethyl-2,6-dioxocyclohex-1-(ylidene)ethyl-(Dde)-OH was coupled to deprotected Fmoc-glycine(Gly)-Sasrin resin using standard coupling procedures [diisopropylcarbodiimide (DIC)/4-hydroxybenzotriazole (HOBt)] (Scheme 1). The lysine template **1** was then derivatized by removing the Fmoc protecting group (20% piperidine in DMF) and then acylated (with either 4-cyanobenzoyl chloride/triethylamine or 4-pentynoic acid or 3,5-di-*tert*-butyl-4-hydroxybenzoic acid/DIC/HOBt) to give the amide derivatives **2**, **5**, and **6**. Intermediates **2** and **6** were then further elaborated by removing Dde by hydrazinolysis and then

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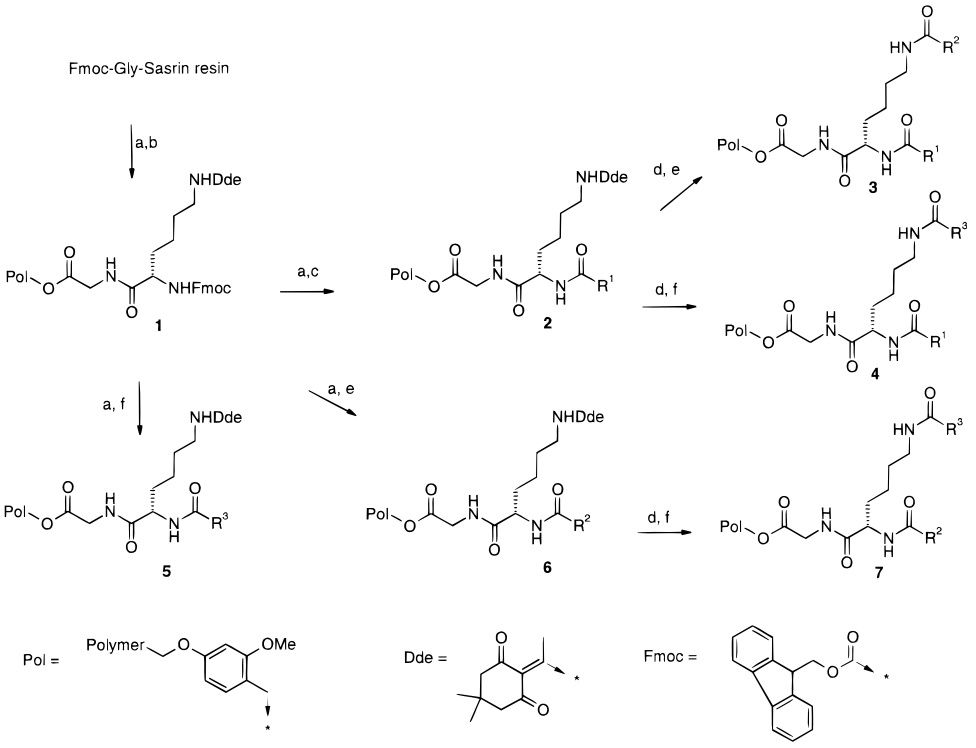
Scheme 1[a]



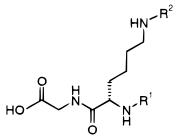
[a] Reagents and conditions: (a) 20% piperidine/DMF; (b) Fmoc-Lys-(Dde)-OH, DIC, HOBt, CH_2Cl_2/DMF; (c) 4-CNPhCOCl, Et_3N, CH_2Cl_2; (d) 4% hydrazine/DMF; (e) 3,5-di-*tert*-butyl-4-hydroxybenzoic acid, DIC, HOBt, DMF; (f) 4-pentynoic acid, DIC, HOBt, DMF.

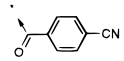
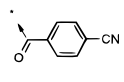
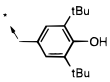
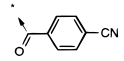
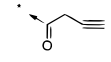
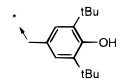
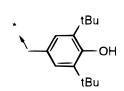
acylated with either pentynoic acid or 3,5-di-*tert*-butyl-4-hydroxybenzoic acid and DIC/HOBt, to give amides **3**, **4**, and **7**, respectively. A sample of each of the polymer-bound compounds (**1** to **7**) was cleaved by hydrolysis [5% trifluoroacetic acid (TFA) in CH_2Cl_2] and the products were analyzed. The expected carboxylic acids, **8** to **14** [structures confirmed by high-resolution mass spectrometry (HRMS), Table 2] were isolated in >95% purity as determined by high-performance liquid chromatography (HPLC).

To mimic the analysis of a mixture, individual beads of **1** to **7** were presented for analysis in a blind manner. The beads, with an average diameter of 45 μm and containing ~0.05 nmol of compound, were flattened into a disk (~300 μm diameter and 2 μm thickness) and then studied by FTIR and Raman microspectrometry.

All six lysine compounds **2** to **7** and the unfunctionalized lysine template **1** were unambiguously identified (Figures 1B and 1C) from their IR spectra by the presence or absence of the diagnostic signal for either the nitrile (2231−2232 cm^{-1}), alkyne (2120−2121 cm^{-1}), or phenolic (3624 cm^{-1}) functional groups. Figure 1A shows an IR spectrum from a single bead of amide **4**. The majority of the peaks in the spectrum originate from the Sasrin resin; however, the following bands can be assigned to the lysine template: 3403 and 3301 cm^{-1} (amide N−H stretch), 1724 cm^{-1} (ester C=O stretch), 1663 cm^{-1} (amide I), and 1269 and 1198 cm^{-1} (ester C−O stretch). The bands at 2232 and 2120 cm^{-1} (the latter of which is very weak) can be assigned to the C≡N and C≡C stretching vibrations. Figures 1B and 1C present expansions of the IR spectra of selected compounds in the regions 3750−3150 and 2300−2000 cm^{-1}, showing the diagnostic signals of the phenol, nitrile, and alkyne groups in the spectra of **2** to **7**.

Table 2. Physical Data of Resin-Cleaved Compounds 8 to 14



compd	R^1	R^2	HPLC retention time (min)[a]	HRMS formula	HRMS M^+
8	Fmoc	Dde	3.91	$C_{33}H_{40}N_3O_7$	590.2917[b]
9		Dde	2.96	$C_{26}H_{33}N_4O_6$	497.2397[c]
10			3.87	$C_{31}H_{41}N_4O_6$	565.3029[d]
11			2.34	$C_{21}H_{25}N_4O_5$	413.1859[e]
12		Dde	2.64	$C_{23}H_{34}N_3O_6$	448.2431[f]
13		Dde	4.31	$C_{33}H_{49}N_3O_7$	599.3605[g]
14			3.61	$C_{28}H_{42}N_3O_6$	516.3052[h]

[a] Purity of all the compounds **8** to **14** was >95%. [b] Calcd for $C_{33}H_{40}N_3O_7$: M^+ 590.2866. [c] Calcd for $C_{26}H_{33}N_4O_6$: M^+ 497.2400. [d] Calcd for $C_{31}H_{41}N_4O_6$: M^+ 565.3026. [e] Calcd for $C_{21}H_{25}N_4O_5$: M^+ 413.1825. [f] Calcd for $C_{23}H_{34}N_3O_6$: M^+ 448.2447. [g] Calcd for $C_{33}H_{49}N_3O_7$: M^+ 599.3571. [h] Calcd for $C_{28}H_{42}N_3O_6$: M^+ 516.3073.

Figure 2A shows a Raman spectrum taken from a small section of a single bead of amide **4**. The spectrum

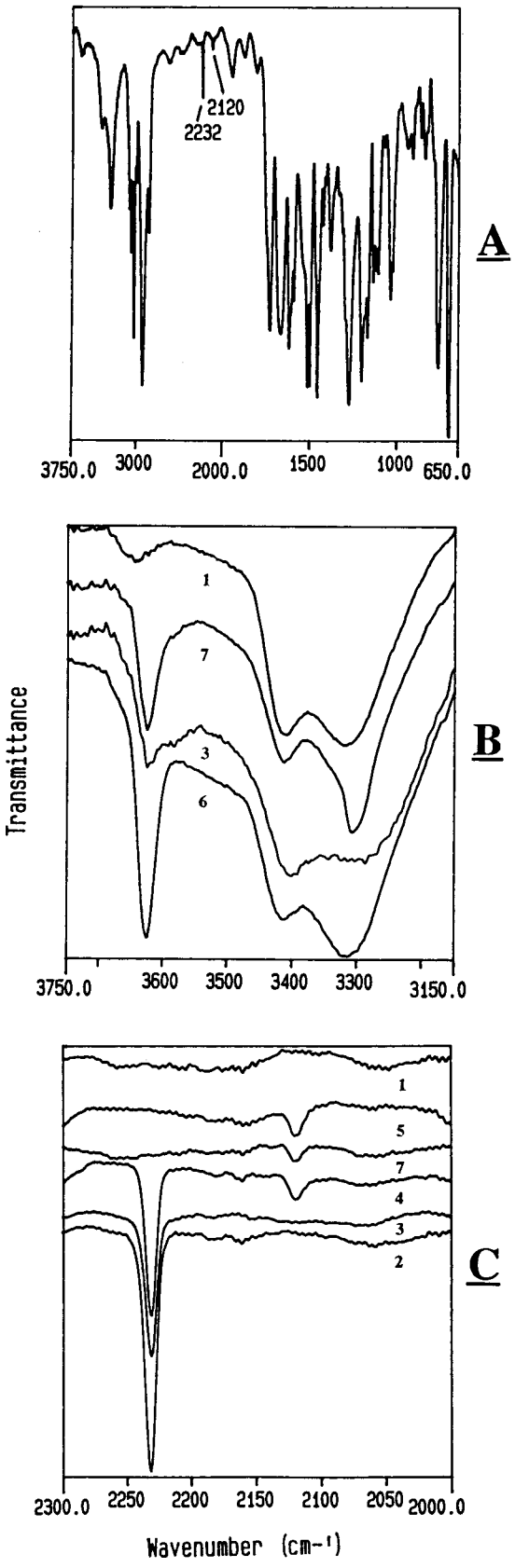


Figure 1. (A) FTIR spectrum of amide **4**. (B) Expansion of the FTIR spectra in the region 3150−3750 cm^{-1} of **1**, **3**, **6**, and **7**, showing the presence or absence of the phenol group. (C) Expansion of the FTIR spectra in the region 2000−2300 cm^{-1} of **1**−**5** and **7**, showing the presence or absence of the nitrile and alkyne groups.

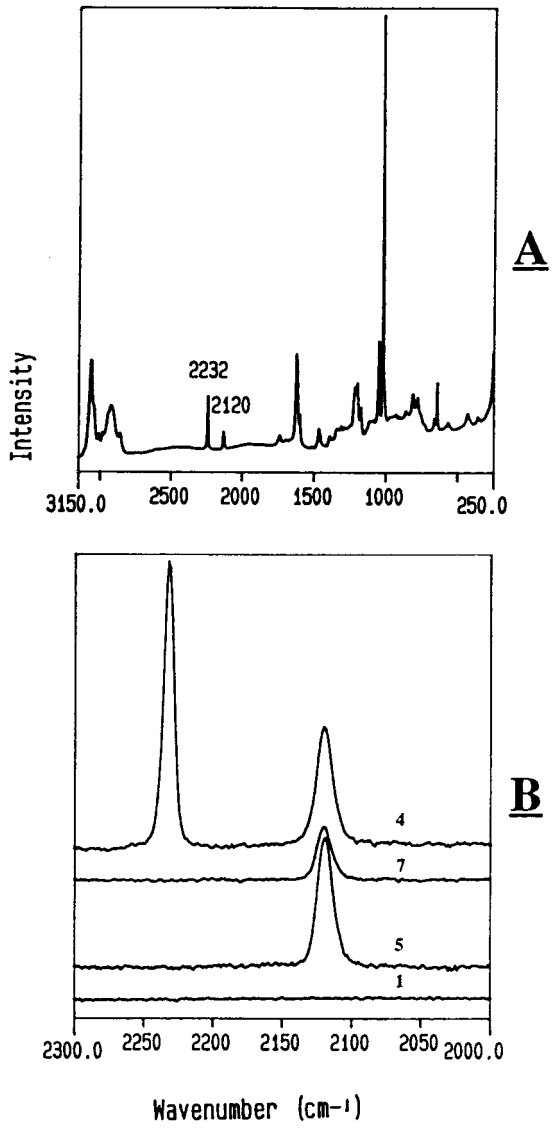


Figure 2. (A) Raman spectrum of amide **4**. (B) Expansion of the Raman spectra in the region 2000−2300 cm^{-1} of **1**, **4**, **5**, and **7**, showing the presence or absence of the nitrile and alkyne groups.

was collected from a 1-μm diameter area of the flattened disk. This corresponds to about one femtomole[11] of compound and demonstrates the high sensitivity of this technique. Although the spectrum is dominated by a ring breathing vibration near 1000 cm^{-1} (Star of David mode) arising from the polystyrene backbone, the stretching bands corresponding to the C≡N and C≡C are clearly identifiable (cf. Figure 1A, where the C≡C stretching band in the IR is almost lost among the interference fringes). The presence of both the nitrile and alkyne groups confirms the structure of the compound attached to the bead as amide **4**. The expanded Raman spectra of beads illustrating C≡C stretching bands are shown in the Figure 2B. From these spectra (and by comparing the respective IR spectra), the compounds attached to the beads selected for analysis can be assigned as amides **4**, **5**, and **7**. In all cases the C≡C stretching band is more

(11) The ratio of the area sampled to the area of the flattened bead is $1/(250)^2$. The amount of material is, therefore, 5×10^{-11} mol (loading per bead)/$(250)^2 = 8 \times 10^{-16}$ mol.

definitive in the Raman spectrum than the corresponding IR spectrum.

In summary, the application of Raman microspectrometry as an analytical tool for solid-phase organic synthesis has been demonstrated. Furthermore, our results show that in addition to being useful as an analytical tool, both FTIR and Raman microspectrometry could also be used to develop a novel encoding strategy for deconvolution of library hits. A possible encoding method would involve the cosynthesis of a set of Raman- and IR-active tags to label the synthetic sequence. Other groups that could be used as IR and Raman tags include: $-C\equiv C-{}^2H$ (2585−2630 cm^{-1}), $-C=C-{}^2H$ (2225−2335 cm^{-1}), $-C-{}^2H$ (2085−2255 cm^{-1}), $-C=C-C\equiv N$ (2215−2240 cm^{-1}), and $-N=N=N$ (2120−2160 cm^{-1}). Additionally, CH_2NO_2 (1375−1390 cm^{-1}), $ArNO_2$ (1310−1355 cm^{-1}), and C−S−S−C (505−515 cm^{-1}) could be used specifically as Raman tags. Unlike most of the other encoding strategies, an advantage of this approach is the ability to access the encoding information while the tags are still attached to the resin. Alternatively, IR and Raman tags could be used to develop a partial encoding strategy where selected library components could be tagged (self-encoding) by using groups that both provide chemical diversity and function as spectroscopic tags. The remaining groups would be identified by other means (e.g., mass spectrometry, color, fluorescence etc.). Using either of these encoding strategies, libraries may be screened while still attached to an appropriate polymer support or after cleavage of arrayed single beads.

Experimental Section

General Aspects. The polymer matrix for all resins used in this work is the copoly(styrene-1% divinyl benzene). Fmoc-Gly-Sasrin resin (loading 0.75 mmol g^{-1}) and Fmoc-Lys-(Boc)-OH were purchased from NovaBiochem and Bachem. HPLC was carried out on a Hewlett-Packard 1100 system equipped with a 4.5 mm i.d. x 75 mm ZORBAX SB−C_{18} reversed-phase column with particle size 3.5 μm. The HPLC was operated with a flow rate of 3 mL/min using 0.1% TFA/H_2O/CH_3CN as eluent with a 0−100% H_2O-to-CH_3CN gradient. A SEDEX 55 Evaporative Light Scattering Detector was used for assessing the purity of the products. High-resolution mass spectra (HRMS) were recorded on a VG 70-SEG mass spectrometer under fast bombardment (FAB) conditions.

Preparation of Fmoc-Lys-(Boc)-Gly-Sasrin Resin (1). Fmoc-Gly-Sasrin resin (0.67 g, 0.5 mmol g^{-1}) was suspended in 20% piperidine in dimethylformamide (DMF, 10 mL). After shaking for 30 min, the resin was filtered and washed with DMF (3 × 5 mL). The resin was then resuspended in a 1:1 mixture of DMF/CH_2Cl_2 (20 mL) and sequentially treated with Fmoc-Lys-(Boc)-OH (1 g, 1.87 mmol), DIC (0.24 g, 1.87 mmol), and HOBt (0.25 g, 1.87 mmol). The mixture was then agitated by argon bubbling for 2 h, and then filtered and washed with DMF (3 × 20 mL) and CH_2Cl_2 (3 × 20 mL). The loading of the lysine-derivatized resin **2** was determined to be 0.61 mmol g^{-1} by Fmoc analysis.[12]

Standard Procedure for Fmoc Group Removal. The resin (0.11 mmol) was washed with DMF (2 × 15 mL). A 20% solution of piperidine in DMF (15 mL) was added with argon bubbling for 20 min. The resin was then filtered and washed with DMF (5 × 15 mL).

Standard Procedure for Acylation with 4-Cyanobenzoyl Chlorides. The resin (0.05 mmol) was washed with CH_2Cl_2 (2 × 15 mL) and then suspended in CH_2Cl_2 (15 mL). The suspension was then treated sequentially with Et_3N (10 equivalents) and a solution of acid chloride (5 equivalents) in CH_2Cl_2 (10 mL). After agitating on an orbital shaker for 2 h, the resin was drained and washed with CH_2Cl_2 (3 × 15 mL), DMF (3 × 15 mL), and methanol (3 × 15 mL).

Standard Procedure for Acylation with Carboxylic Acids (3,5-di-*tert*-Butyl-4-hydroxybenzoic Acid or 4-Pentynoic Acid). The resin (0.05 mmol) was suspended in DMF (15 mL) and then treated sequentially with 5 equivalents each of a carboxylic acid, DIC, and HOBt. After shaking for 18 h, the resin was washed with DMF (3 × 15 mL) and methanol (3 × 15 mL).

Standard Procedure for Dde Group Removal. The resin (0.05 mmol) was suspended in 4% anhydrous hydrazine in DMF (15 mL). The suspension was then shaken for 10 min and then washed with DMF (3 × 15 mL) and MeOH (2 × 15 mL).

Standard Procedure for Resin Cleavage of Lysine Derivatives 1−7. The polymer-bound compounds **1** to **7** were separately cleaved by suspending 0.02 g of each of the resins in 5% TFA in CH_2Cl_2 (1 mL) for 0.5 h. The resins were then filtered and washed with CH_2Cl_2 (3 × 1 mL). The filtrates were then evaporated under reduced pressure to give the products **8** to **14**. The cleaved products were then analyzed (Table 2) by HPLC and HRMS.

FTIR Microspectroscopy. Individual beads (manipulated with microtweezers under a microscope) were flattened using a Spectra-Tech μ-Sample Plan compression cell fitted with diamond windows. This flattening was necessary to allow adequate transmission of IR radiation at all frequencies. Following separation, the window with the adhering bead was transferred to a Spectra-Tech IR−Plan microscope coupled to a Perkin-Elmer 1760 FTIR spectrometer. Remote apertures were used to define an area 100 μm in diameter, from which 256−1024 scans were signal-averaged at a resolution of 4 cm^{-1} using a medium Norton−Beer function for apodization. Spectra were "ratioed" against a background recorded through a clear section of the window using the same apertures. The microscope and spectrometer transfer optics were purged with dry air.

Raman Microspectroscopy. Spectra were obtained using a Renishaw Ramascope and a He−Ne laser emitting at 633 nm (power at sample location ≈3 mW). Spectra were acquired over a limited (≈700 cm^{-1}) window (static grating mode) using an exposure time of 200 s. Continuous extended scanning[13] was used to obtain spectra covering the full spectral range. Spectral resolution was better than 4 cm^{-1}. Although good quality spectra were obtained from individual beads without sample preparation, it was found that flattening beads (on a glass slide using an aluminum roller) produced a significant improvement in baseline linearity.

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