Multistep Synthesis on SU-8: Combining Microfabrication and Solid-Phase Chemistry on a Single Material

Gabriel Cavalli,[†,#] Shahanara Banu,[†] Rohan T. Ranasinghe,[†] Graham R. Broder,[†] Hugo F. P. Martins,[†] Cameron Neylon,[†,‡] Hywel Morgan,[§] Mark Bradley,[||] and Peter L. Roach*,[†]

School of Chemistry, Highfield Campus, University of Southampton, Southampton SO17 1BJ, U.K., Isis Pulsed Neutron and Muon Facility, Rutherford Appleton Laboratory, Chilton, Didcot OX11 0QX, U.K., School of Electronic and Computing Sciences, Highfield Campus, University of Southampton, Southampton SO17 1BJ, U.K., and School of Chemistry, The University of Edinburgh, Joseph Black Building, The King's Buildings, West Mains Road, Edinburgh, EH9 3JJ, U.K.

Received June 12, 2006

SU-8 is an epoxy-novolac resin and a well-established negative photoresist for microfabrication and microengineering. The photopolymerized resist is an extremely highly crosslinked polymer showing outstanding chemical and physical robustness with residual surface epoxy groups amenable for chemical functionalization. In this paper we describe, for the first time, the preparation and surface modification of SU-8 particles shaped as microbars, the attachment of appropriate linkers, and the successful application of these particles to multistep solid-phase synthesis leading to oligonucleotides and peptides attached in an unambiguous manner to the support surface.

Introduction

SU-8 is a novolac-epoxy resin well established in the field of microfabrication and micropatterning as a negative photoresist.[1−3] Commercially available formulations (Shell Chemical, MicroChem) provide macromonomer **1** (Scheme 1) dissolved in γ-butyrolactone at different concentrations.[1] These different formulations are spin-coated onto a wide range of substrates giving rise to film thicknesses in the range of 1−100 μm, depending on the viscosity of the original solution and the spin speed.[1−3] They also contain a photoacid to initiate polymerization of the epoxide groups upon UV exposure.[1−3] The resulting polymerized resin is a highly crosslinked polymer, the hardness of which depends on the time and energy of the UV exposure and the postexposure baking times and temperatures.[1−3] Photolithographic masks are used to pattern the SU-8, producing well-defined structures with high aspect ratios (ratio of height to width) and excellent mechanical properties.[2,3] Thus, SU-8 has been successfully fabricated into a range of microstructures including microfluidic structures,[4,5] molds and masters for microembossing,[6,7] probes for microscopy,[8,9] and biosensors.[10] Because of its outstanding performance in microfabrication, optical transparency in the visible range, and excellent physical and chemical stability, SU-8 has recently attracted attention for use in bioanalytical applications as a support for the direct attachment of biomolecules.[11−16] After microfabrication, residual surface epoxy groups are suitable to act as reactive sites for surface functionalization.[11,12]

Unpolymerized SU-8 has been used in chip technology for DNA hybridization assays coated on glass.[13−15] More recently, photopolymerized and structured SU-8 has been used for DNA hybridization.[16] Immobilization of DNA probes was carried out by spotting either amino-modified or unmodified oligonucleotides on the surface of SU-8 films and structures microfabricated through photolithography. This resulted in an uncertain mode of chemical attachment and indeterminate orientation of oligonucleotide probes on the SU-8 surface.[16] In this paper, we describe, for the first time, the preparation and surface modification of SU-8 particles shaped as microbars, the attachment of appropriate spacers in combination with linkers, and the successful application of these particles to multistep synthesis leading to oligonucleotides and peptides attached in an unambiguous manner to the support surface. While SU-8 is not envisioned as a direct competitor for the traditional supports routinely used for solid-phase synthesis, its establishment in microfabrication coupled with the feasibility of carrying out multistep synthesis suggest myriad interesting applications in bioanalytical sciences including, for example, microfabricated encoded carriers, combinatorial biomolecular arrays, and microfluidic devices with built-in synthetic molecular probes.

Results and Discussion

Fabrication of SU-8 Microparticles. The fabrication of SU-8 microparticles suitable for multistep synthesis required significant optimization. The mask for the photolithographic

* To whom correspondence should be addressed. E-mail: P.L.Roach@soton.ac.uk.

[†] School of Chemistry, University of Southampton.

[‡] Isis Pulsed Neutron and Muon Facility, Rutherford Appleton Laboratory.

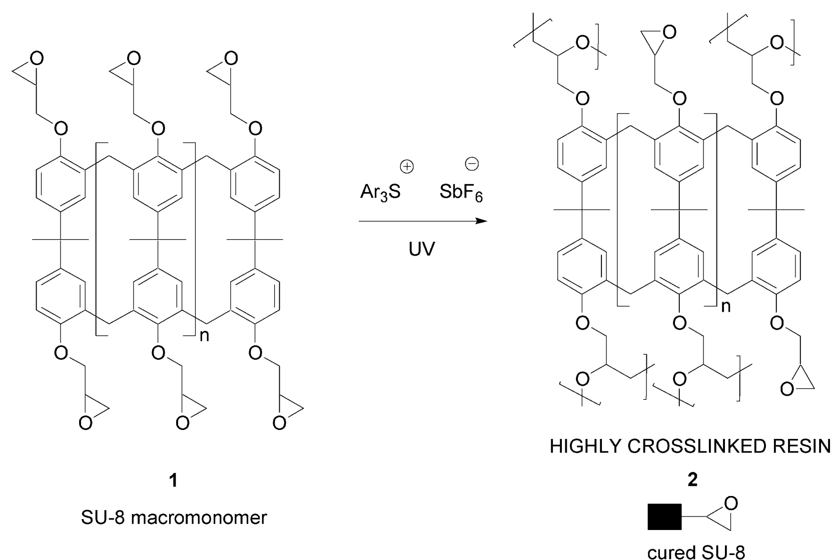
[§] School of Electronic and Computing Sciences, University of Southampton.

[||] School of Chemistry, The University of Edinburgh.

[#] Current address: School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, U.K.

10.1021/cc060079p CCC: $37.00 © 2007 American Chemical Society
Published on Web 04/07/2007

Scheme 1. Polymerization of SU-8



process was designed to produce microparticles (cross section = 20 × 10 μm) comparable in size to the beaded polymer supports used in conventional multistep solid-phase synthesis. For a typical film height (3−4 μm), this yields 10 mg of particles/wafer. The microfabrication process exposes the particles to a wide range of physical and chemical conditions, some of which could potentially modify the surface properties of the particles and render them unsuitable for further chemistry.[1−3] Therefore, an important feature of this work was to determine whether the fabricated microparticles remained suitable for synthetic chemistry.

SU-8 microparticles were prepared by conventional photolithography (Figures 1 and 2a). To allow the release of microparticles from the substrate after microfabrication, a sacrificial layer was incorporated in the process, as shown in Figure 1. The selection of the sacrificial layer was based on good adhesion properties toward SU-8, ease of handling and simplicity of etching process.[17−20] The last variable is critical and requires that the sacrificial layer is efficiently and rapidly removed, releasing the SU-8 particles into suspension without compromising the presence of residual surface epoxy groups to be used for functionalization.

The use of aluminum as a sacrificial layer was investigated.[18] A thin layer was evaporated onto a substrate, followed by spin coating with a Ti primer prior to SU-8 processing, which resulted in excellent adhesion of the SU-8. After photopolymerization and a postexposure bake, the non-exposed areas were developed, and the sacrificial layer was etched. Clean etching of the aluminum layer was successful using an ultrasonic bath with commercial developer MF 319 (tetramethylammoniumhydroxide solution, TMAH, supplied as a 2.2% w/v solution in water). In all cases, the released SU-8 microparticles were collected by centrifugation, washed with methanol, and then dried under vacuum to produce a dry SU-8 support (Figure 2b). The yield of particles was 8 mg/wafer (80%). The suitability of these microparticles for peptide and oligonucleotide synthesis was then investigated.

Chemistry on SU-8. Although optimization of the microfabrication was essential for optimal synthesis on SU-8, chemical functionalization was first studied using large pieces of SU-8 prepared on glass. Films of SU-8 were produced this way by overexposure under UV and grinding the final film resin (100 μm thick) to small pieces (visually not more than 1 mm). This simpler process rapidly provided large quantities of material (SU-8, **3**) for preliminary studies on the functionalization chemistry as shown in Scheme 2. It

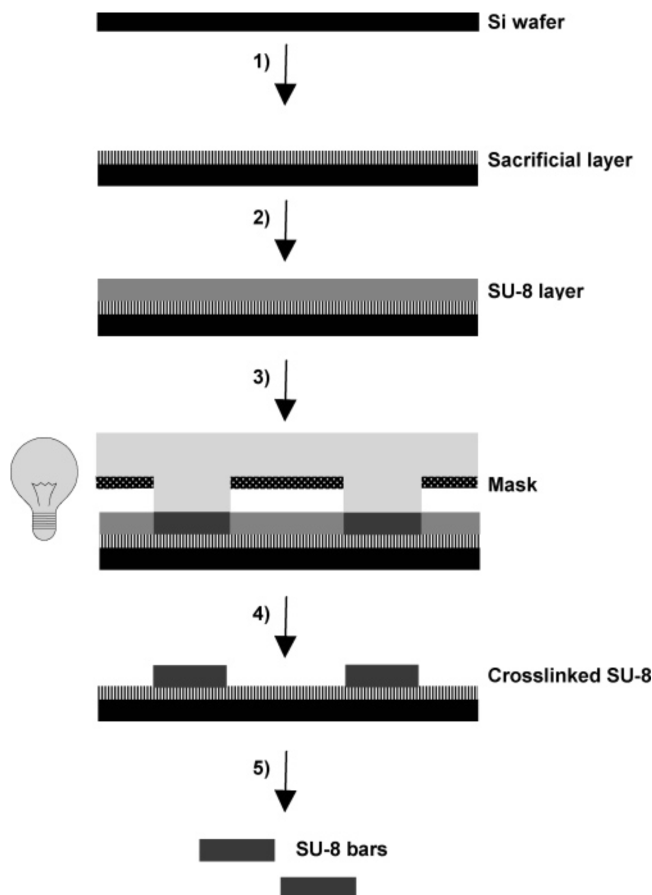


Figure 1. Microfabrication of SU-8 particles by photolithography: (1) add sacrificial layer (Al), (2) add SU-8 and bake, (3) expose to UV and bake, (4) develop non-crosslinked SU-8, and (5) etch sacrificial layer.

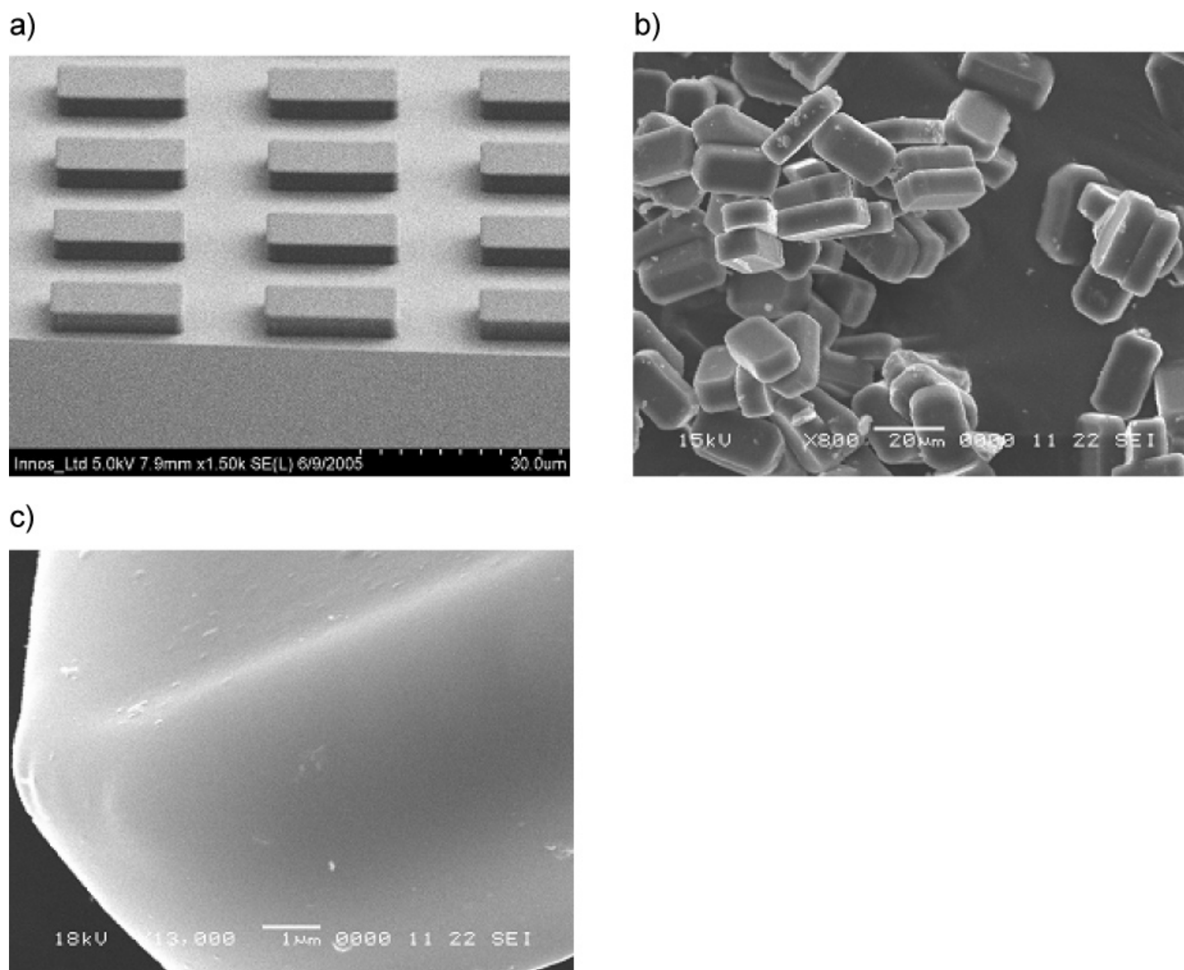
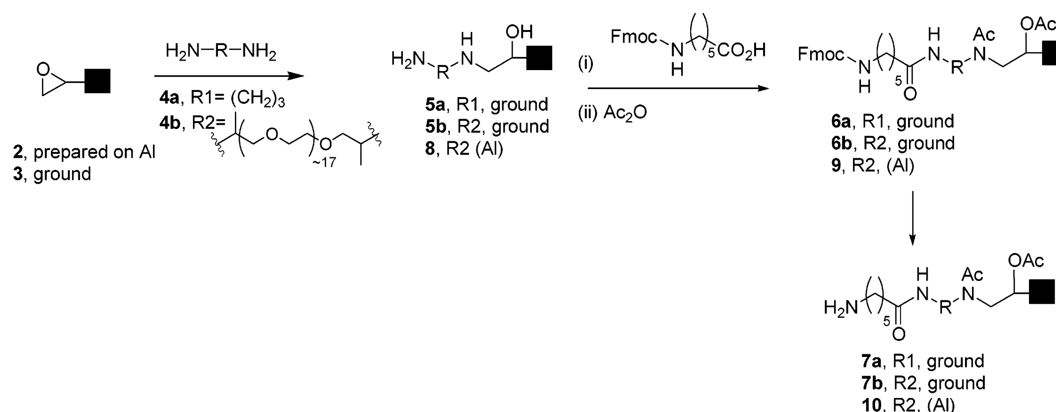


Figure 2. Scanning electron microscopy analysis of SU-8 microparticles prepared on an Al sacrificial layer: (a) SU-8 particles on Si-wafer after microfabrication (size of bar = 30 μm), (b) SU-8 particles after functionalization with Jeffamine (size of bar = 20 μm), and (c) higher magnification of an SU-8 particle.

Scheme 2. Functionalization of SU-8

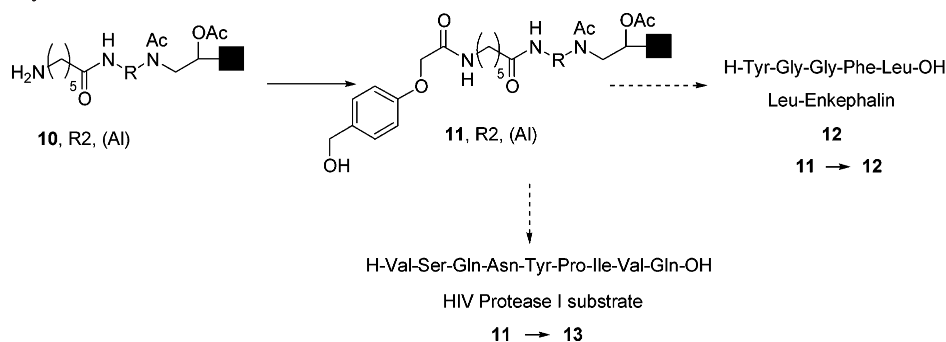


was anticipated that the straightforward chemistry of functionalization could subsequently be applied to well-defined and carefully microfabricated particles. The residual epoxy groups present on the surface of SU-8 were reacted with bisamines to introduce free amino groups, selected (Scheme 2) using a similar procedure to that applied to other epoxide-containing polymer supports such as GMA (glycidyl methacrylate supports).[21,22] 1,3-Diaminopropane (**4a**) and Jeffamine$_{800}$ (**4b**) were selected as suitable amines and provided spacers of different lengths between the SU-8 surface and the primary amine. The conditions resulting in the highest loading levels were found to be overnight stirring in acetonitrile at 65 °C. Test washes with hot and cold acetonitrile and analysis of the washings suggested that the excess bisamine was thoroughly removed by a series of simple washing/centrifugation cycles with acetonitrile at room temperature, although the possibility of permanent physical entrapment of the bisamines in the polymer network cannot be excluded. The loading levels of the amino supports (**5**) were approximately quantified using the ninhydrin test (Table 1). The use of 1,3-diaminopropane (**4a**) resulted in a loading level of 20 μmol/g, while Jeffamine$_{800}$ (**4b**) gave a

Table 1. Functionalization of SU-8 and Solid-Phase Synthesis of Leu-Enkephalin.

reaction type/process stage	loading levels of supports (method of measurement, observations)		
starting material	**3**	**3**	**2**
functionalization with bisamine	**5a**, 20 ± 3 μmol/g (ninhydrin)	**5b**, 9 ± 3 μmol/g (ninhydrin)	**8**, 15−25 μmol/g (ninhydrin)
attachment of Fmoc-aminohexanoic acid	**6a**, 4 ± 2 μmol/g (Fmoc)	**6b**, 9 ± 2 μmol/g (Fmoc)	**9**, 15−19 μmol/g (Fmoc)
deprotection of amino groups	**7a**	**7b**	**10**, 16−18 μmol/g (ninhydrin)
attachment of HMPA linker			**11**
attachment of first amino acid (Leu)			15 μmol/g (Fmoc)
attachment of Phe			single coupling
attachment of Gly			single coupling
attachment of Gly			single coupling
attachment of Tyr			single coupling
peptide cleavage			**12**, 0.5 mg crude isolated (expected 0.5 mg, but only 5% estimated from HPLC trace). ESI-MS: found 556.5 (MH^+) (expected 556.6)

Scheme 3. Peptide Synthesis on SU-8



loading level of 9 μmol/g. The lower loading levels observed with Jeffamine$_{800}$ (**4b**) may be rationalized in terms of the reduced accessibility of epoxide groups on the polymer to this bulkier nucleophile.

The resultant amino groups were then coupled to *N*-Fmoc-6-aminohexanoic acid using standard carbodiimide/*N*-hydroxybenzotriazole (HOBt) chemistry.[23] Quantitation of this reaction by release of the Fmoc group with piperidine in *N,N*-dimethylformamide (DMF) showed that while Jeffamine-derived aminosupport **5b** led to quantitative coupling (relative to the initial loading levels), 1,3-diaminopropane-derived support **5a** showed much lower coupling efficiency (Table 1).[23] The higher initial coupling of 1,3-diaminopropane and lower subsequent yields may both be rationalized in terms of its size. The initial reaction is likely to be efficient because the smaller nucleophile is able to access more epoxide groups, but the resultant amine groups may be slower to react because of their proximity to the surface. For this reason, the use of 1,3-diaminopropane was abandoned, and all further synthetic studies made use of Jeffamine for the functionalization of the SU-8 resin.

Having demonstrated the feasibility of functionalizing SU-8 on this model material, we carried out further studies with microfabricated SU-8 particles. Microparticles prepared using aluminum (**2**) as sacrificial layers were treated with Jeffamine (**4**) at 65 °C in acetonitrile overnight. The ninhydrin assay of the amino particles (**8**) showed an amino group loading of 22 μmol/g (Table 1).

The initial functionalization was extended by attachment of a second spacer, *N*-Fmoc-6-aminohexanoic acid, followed by capping of the residual nucleophiles on the surface of the particles (Scheme 2). The efficiency of this coupling reaction to form **9** (Scheme 2) could be determined by cleaving the Fmoc group from a small aliquot of particles, and this showed that the reaction proceeds quantitatively (within experimental error).

Peptide Synthesis. To test the performance of SU-8 in multistep synthesis, solid-phase peptide synthesis was chosen as a well-established methodology,[24] and the resultant peptides are attractive targets in a myriad of biotechnological assays and applications.[25] Leucine-enkephalin was selected as a simple test sequence. It is a pentapeptide (Tyr-Gly-Gly-Phe-Leu) that is routinely used as a model for testing materials as supports for synthesis.[26−28] To permit Fmoc chemistry, 4-hydroxymethylphenoxyacetic acid (HMPA) was attached as a linker using carbodiimide/HOBt chemistry at room temperature[23] (Scheme 3). The first amino acid (*N*-Fmoc-Leu) was coupled onto the resin using carbodiimide/*N,N*-dimethyl-4-aminopyridine (DMAP) at room temperature.[23] A quantitative Fmoc test showed efficient loading of

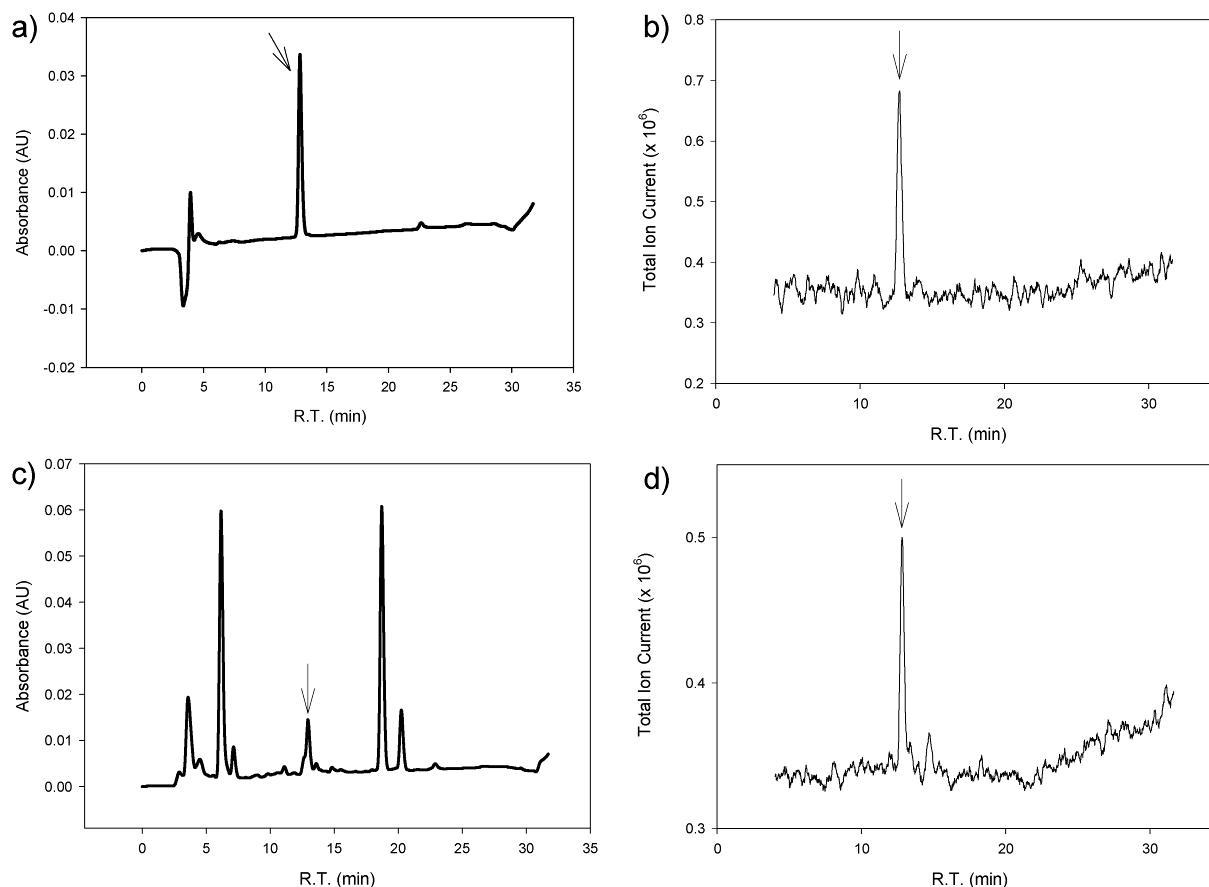


Figure 3. HPLC-MS analysis of Leu-enkephalin: (a) HPLC of standard Leu-enkephalin, (b) total ion current (TIC) standard of Leu-enkephalin, (c) HPLC of **12a** (synthesis on SU-8), and (d) TIC of **12a** (synthesis on SU-8). The HPLC trace corresponds to UV detection at 215 nm. Total ion currents are reported in arbitrary units. The arrow indicates the retention time of the standard peptide.

the first amino acid (Table 1). Subsequent couplings were carried out using (*N*,*N*,*N*′,*N*′-tetramethyl-*O*-benzotriazol-1-yl)uronium tetrafluoroborate (TBTU) as the coupling reagent.[23] After the synthesis, the peptides were cleaved from the support using TFA/phenol (98:2). The available quantities of microparticles limited the synthesis to a very small scale (50 mg of SU-8 particles, 850 nmol scale synthesis), and this scale restricted the range of techniques available for product characterization. However, HPLC-MS analysis required very small quantities of material and allowed comparison of the synthetic peptide samples with a commercial sample of Leu-enkephalin (Figure 3a and b). SU-8 (**11**) produced Leucine-enkephalin (**12**) which was analyzed by HPLC-MS. The UV trace (Figure 3c) shows several peaks, although only one was associated with the peak in the total ion current trace (Figure 3d). Either the impurities observed in the UV trace are peptides that do not ionize well, or they are not peptidyl in origin. A potential source of the impurities is the deprotection step. Impurities may be material leached from the SU-8 or contaminants from the cation scavenger (phenol). Further experiments will be required to identify these impurities and hence develop suitable reaction conditions to minimize them. The yield of product (**12a**) with respect to the loading of the first amino acid was estimated to be 5% by chromatographic comparison with a commercial standard (Figure 3a and c). This yield may indicate low stepwise coupling efficiency but may simply highlight the practical difficulties in isolating polar compounds such as peptides from manual nanomolar scale syntheses. A commercial sample of Leu-enkephalin showed identical mass spectrometric and chromatographic properties to those found for **12a**. This initial study suggested that SU-8 microparticles provide a viable support for solid-phase peptide synthesis.

To confirm this, the synthesis of a larger peptide was then explored. A nonapeptide (HIV protease 1 substrate) was chosen as the target (**13**, Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln).[29−31] Nonapeptide **13** was synthesized on SU-8 (**11**) (Scheme 3). Because peptide **13** was not commercially available, a synthetic standard of **14** was prepared by standard SPPS using standard PS resin with a Wang linker, **15**. The synthesis of **13** proceeded smoothly requiring only single couplings.[23] The product peptides were analyzed by HPLC-MS after cleavage, and the results are shown in Figure 4 and Table 2. The yield of crude peptide **13** was higher than in the previous case (40% by weight), but the crude material contained the required peptide (<25% by UV), together with several impurities that did not give readily identifiable ions in the mass spectrometer. As expected the crude product from conventional SPPS, **14**, was relatively pure with a single minor impurity. The MS of the impurity was consistent with a dehydration product (possibly dehydration of Asn or Gln) (Figure 4c and d, Table 2). The nonapeptide synthesized on SU-8 microparticles (**13**) contained the same minor impurity. The identity of the sample prepared on SU-8 was further confirmed by co-elution of the crude **13** with HPLC purified

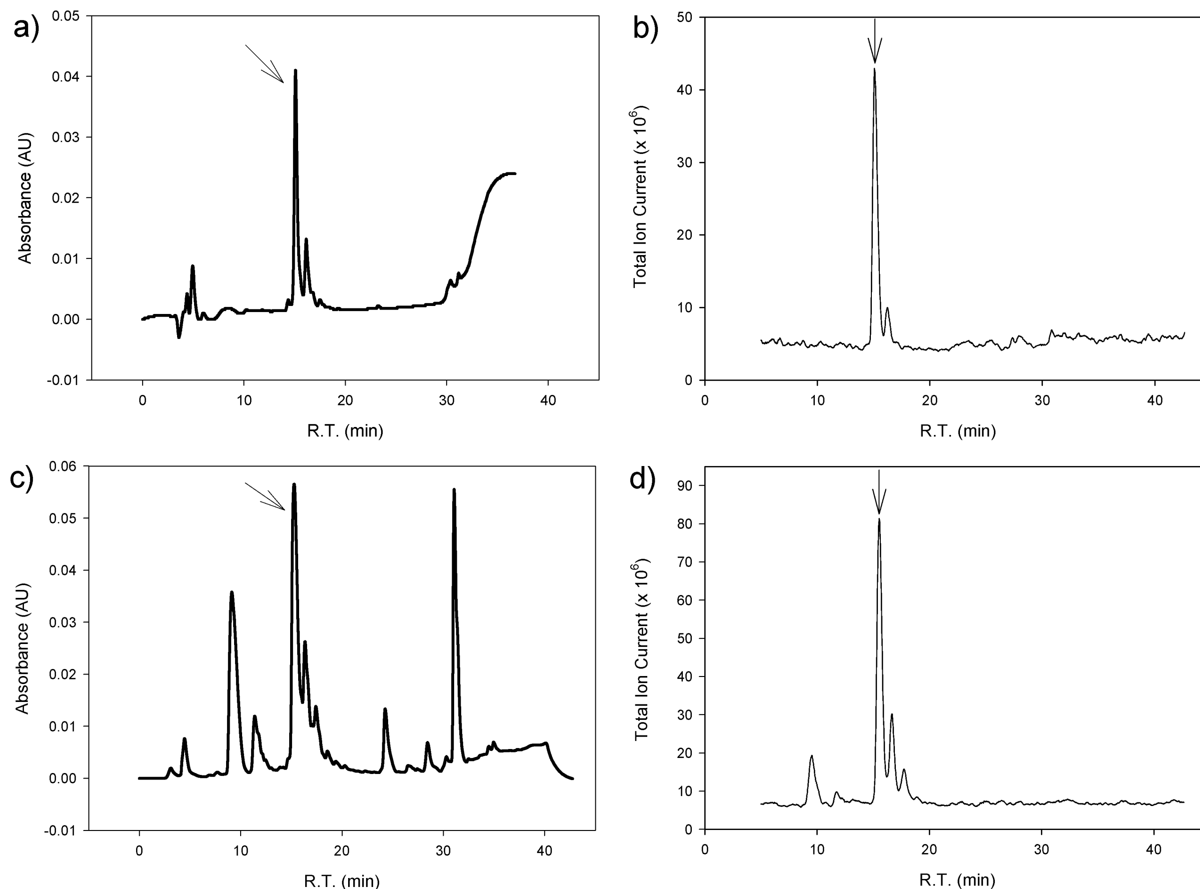


Figure 4. HPLC-MS analysis of HIV protease I substrate: (a) HPLC of **14** (Wang resin product), (b) TIC of **14** (Wang resin product), (c) HPLC of **13** (SU-8 product), and (d) TIC of **13** (SU-8 product). HPLC trace corresponds to UV detection at 215 nm. Total ion currents are reported in arbitrary units. The arrow indicates the target material.

Table 2. Solid-phase Synthesis of HIV Protease I Substrate

HIV protease I substrate (support)	yield	overall purity of nonapeptide	overall elimination product	nonapeptide with respect to other peptides (ESI-MS)	elimination product with respect to other peptides (ESI-MS)	main peptide impurity[c] (ESI-MS)
13 (SU-8 11)	40%[a]	29%	11%	58% (found 1047.6 (MH$^+$), expected 1048.2)	15% (found 1029.5 (MH$^+$), expected 1030.2)	15% (found 610.0 possible Val-Ser-Gln-Asn-Tyr (MH$^+$), (expected 610.6)
14 (Wang 15)	87%[b]	67%	23%	88% (found 1046.8 (MH$^+$), expected 1048.2)	12% (found 1028.8 (MH$^+$), expected 1030.2)	0%

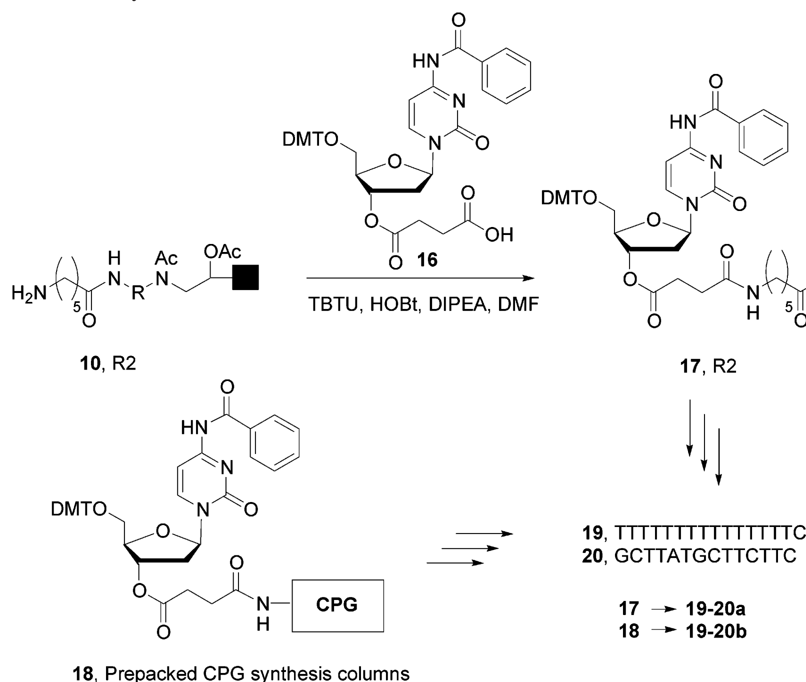
[a] Estimated from integration of HPLC trace compared to calibrated data with pure **14**. [b] Estimated from weight of crude. [c] Excluding elimination product.

14. The lack of truncated failure sequences indicated that the stepwise coupling efficiencies were high.

The objective of this study was to establish the feasibility of solid-phase peptide synthesis on SU-8. These peptide syntheses were carried out on a very small scale, limiting the characterization that was possible. The observed data are consistent with the formation of the required peptide, although after cleavage from the SU-8 support, both peptides were contaminated with impurities. The lack of readily identifiable mass ions for the impurities suggests they are not simple peptidyl products closely related to the target sequence: they do not give mass ions corresponding to simple deletion sequences, dehydration, or alkylation products. Full analysis of these impurities will require a more detailed study, particularly of the stability of the SU-8 matrix during the acid deprotection step.

Oligonucleotide Synthesis. To demonstrate the flexibility of SU-8 as a support for solid-phase synthesis, oligonucleotides were targeted through the phosphoramidite approach.[32,33] Amino-SU-8 (**10**) was functionalized with succinimidyl nucleoside **16** to produce amide **17** (Scheme 4). The loading level was measured by release of the dimethoxytrityl cation under acidic conditions and was found to be 25 μmol/g.[32] SU-8 (**17**) was used in the synthesis of the following oligonucleotides: $T_{15}C$ (**19a**) and GCTTATGCT-TCTTC (**20a**). These sequences were selected as simple tests

Scheme 4. Oligonucleotide Synthesis on SU-8



of the synthetic methodology and not from natural genomic sequences. In parallel to this synthesis, the same oligonucleotide sequences were also prepared on pre-packed CPG columns (**18**, 1000 Å pore size, 50 nmol scale from Bioautomation), producing oligonucleotides **19b** and **20b**. For the synthesis, an automatic MerMade 192 synthesizer (Bioautomation) was used following manufacturer protocols optimized for the synthesis on column CPG (**18**) and performing double phosphoramidite couplings on the SU-8 supports. After conventional synthesis (DMT off) and cleavage of the oligonucleotides from the support, the oligonucleotides were characterized by capillary electrophoresis (shown in Figure 5) and MALDI-TOF MS.[34−36] SU-8 (**17**) produced oligonucleotides **19a** and **20a** which were less pure than those obtained from CPG (**19b** and **20b**) as shown in Figure 5 and Table 3. However, mass spectrometry and capillary electrophoresis indicate that the major products are the required oligonucleotides. These results indicate that SU-8 is suitable for the synthesis of short oligonucleotides suitable for hybridization probes or PCR

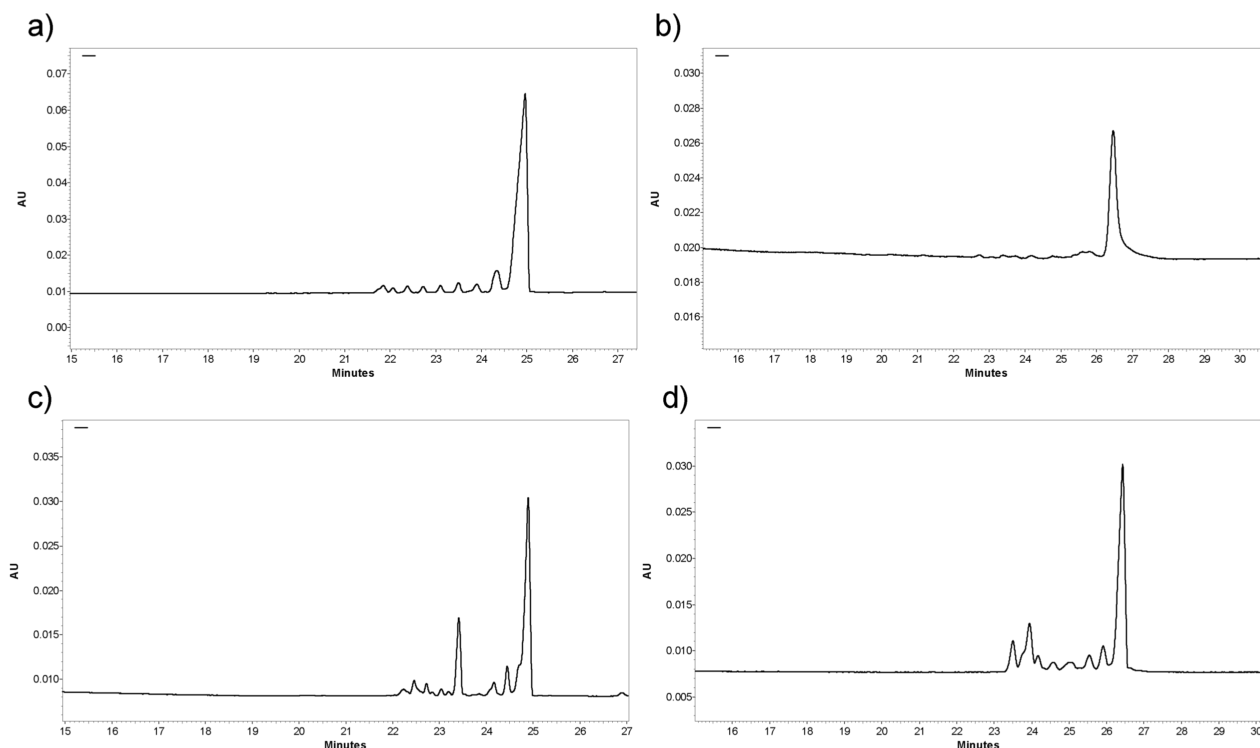


Figure 5. Capillary electrophoresis of oligonucleotides: (a) sequence **19** synthesized on **18** (column CPG), (b) sequence **20** synthesized on **18** (column CPG), (c) sequence **19** synthesized on **17a** (SU-8), and (d) sequence **20** synthesized on **17a** (SU-8).

Table 3. Solid-Phase Synthesis of Oligonucleotides on SU-8 and Comparison with CPG

	oligonucleotide sequence (support)	results		
		purity of target oligonucleotide (%)[a]	mass by MALDI-TOF (expected)[b]	principal impurity (%) (length in bases)[a]
1	**19a (17a)**	47	4791.5 (4791.2)	15 (13)
2	**19b (18)**	77	4791.1 (4791.2)	6 (15)
3	**20a (17a)**	56	4196.6 (4196.8)	20 (5)[c]
4	**20b (18)**	91	4196.6 (4196.8)	3 (11)

[a] Estimated from CE data. [b] Corresponds to $(MH)^+$. [c] Estimated from HPLC data (not shown).

primers, but further optimization of the oligonucleotide synthesis protocols will be required for efficient synthesis of longer sequences (30 or more residues).

Conclusions

Fabrication of releasable SU-8 microparticles on silicon wafers was achieved using standard photolithography techniques. In this process, aluminum proved to be a suitable sacrificial layer for the release of bars from the silicon substrate. The use of basic conditions led to microparticles that could be utilized in subsequent chemistry.

The use of SU-8, a well-established material for microfabrication, has been demonstrated as a feasible support for peptide and oligonucleotide synthesis. SU-8 is not envisioned as a competitor for established supports, but these important results demonstrate the potential for the combination of microfabrication of SU-8 microstructures, followed by functionalization via multistep solid-phase synthesis. This very useful combination of properties is suitable for application in a wide range of bioanalytical systems and processes.

Experimental Section

Microfabrication of SU-8 Particles. The photoresist SU-8 (SU-8-2, SU-8-25, and SU-8-50) and developers (Microposit EC and propylenglycol methyl ether acetate, PGMEA) were supplied by Chestech Ltd, U.K. Microposit MF-319 (tetramethylammoniumhydroxide, TMAH, 2.2% w/v solution in water) was obtained from Shipley Europe Ltd. The universal Ti primer and single-sided polished 4 in. (100 mm) silicon wafers (thickness = 525 ± 25 μm) were purchased from Microchem Corp. and Si-Mat Silicon Materials, Germany, respectively. A Headway Research spinner and a SUSS Microtech Mask Aligner MA6 (lamp HG 1000 DC) were used for spinning and exposing the SU-8. The photomask (dark field) was designed to produce rectangular bars (20 × 10 μm) separated by a 10 μm spacing. It was generated using a CAD package (L-Edit 11.0) and printed on a glass–chromium photomask by Compugraphics International.

Crosslinked SU-8 for Functionalization Studies. SU-8-50 (1 mL) was spun onto thin objective microscope cover glass slides at 2000 rpm for 2 min. The slides were soft-baked at 95 °C for 30 min and exposed to UV light (100 W UV–vis bulb, cutoff filter 365 nm) for 5 min. The exposed slides were soft-baked at 95 °C for 30 min, and the SU-8 was easily lifted off the glass slides by gently bending the slides. The crosslinked SU-8 films were ground to particle sizes not bigger than several hundred micrometers (visually less than 1 mm).

SU-8 Microfabrication by Photolithography. Standard photolithographic methods were used to fabricate the SU-8 micro-particles onto silicon wafers (4 in.) (Figure 1). A six-step process was optimized as follows: (1) selection and coating of sacrificial layer, (2) optimization of adhesion of SU-8, (3) spin coating of SU-8, (4) photolithography, (5) development, and finally, (6) the lift off process. Silicon wafers were cleaned by immersion in fuming nitric acid for 20 min and then rinsed twice in water. The acid-cleaned wafers were then spun dry and baked at 200 °C in a convection oven overnight. A 50 nm thick sacrificial layer of Al was coated onto pre-cleaned silicon wafers using an E-Gun evaporator; the coated wafers were washed with acetone and isopropanol and blow dried. The wafers were then baked at 200 °C for 1 h to ensure dryness, and a universal Ti primer (sufficient to coat the entire wafer, ~3 mL) was then spin coated onto the Al-coated wafers at 2500 rpm for 30 s. The resultant wafers were then baked at 120 °C for 10 min in an oven.

SU-8-2 was spin coated onto pre-cleaned and primed wafers using the spin cycle started from a spread cycle at 500 rpm for 5 s at an acceleration of 100 rpm/s, followed by a final cycle at 1500 rpm for 30 s at an acceleration of 300 rpm/s. A similar process was applied for spin coating SU-8-5, except that the final cycle was at 2500 rpm. After application of the SU-8 layer, the wafers were soft baked at 65 °C for 3 min, and then the temperature was increased at 4 °C/min to 95 °C; then the temperature maintained for 5 min for SU-8-2 and 10 min for SU-8-5.

The SU-8-coated wafers were exposed to 365 nm light, optimized by variation of the exposure time from 3 to 10 s with increments of 1 s (at a rate of 20.1 mW/cm^2) with exposure doses between 90 and 110 mJ/cm^2. After exposure, the wafers were baked at 65 °C for 1 min, and then the temperature was increased at 4 °C/min to 95 °C for 1 min for SU-8-2 and 3 min for SU-8-5, respectively. The wafers were then left to cool to room temperature. The wafers were developed in PGMEA for 2 min with agitation, then thoroughly rinsed with isopropyl alcohol, and blow dried. The sacrificial layer was removed by sonication of the wafers in TMAH (2.2% w/v solution in water, Microposit MF-319) at room temperature for 10 min. The released microparticles were collected by centrifugation (13 000 rpm for 1 min), then washed in methanol (1 mL × 8), and dried under vacuum at room temperature for 4 h. The yield of isolated particles was typically 8 mg/wafer (80%).

Chemistry on SU-8. All reactions were carried out in microcentrifuge tubes (1.5 mL), and separation of the support

from solutions was carried out by centrifugation at 13 200 rpm for 1−3 min in an accuSpin Micro microcentrifuge (Fisher Scientific). Ninhydrin, Fmoc, and trityl tests were carried out by UV spectrophotometry as reported in the literature.[33,37,38]

Functionalization of Fragmented SU-8. SU-8 (100 mg) was treated with $Jeffamine_{800}$ (500 mg, neat) and acetonitrile (500 μL), and the mixture was heated to 65 °C in an oven overnight. The support was washed with acetonitrile (7 × 800 μL), followed by methanol (7 × 800 μL), and dried under vacuum at room temperature for 4 h to yield **8a** and **8b**. *N*-Fmoc-6-aminohexanoic acid (5.0 mg, 14 μmol) was dissolved in DMF (100 μL), and *N*,*N*′-diisopropylcarbodiimide (DIC) (2 μL, 13 μmol) was added. The mixture was shaken for 8 min at room temperature; HOBt (2 mg, 15 μmol) was added, and the mixture was shaken for 5 min at room temperature. The mixture was added to amino SU-8 (1.50 μmol based on free $-NH_2$ groups) and suspended in DMF (300 μL), and the mixture was heated to 60 °C for 1 h. The support was washed with DMF (7 × 800 μL), followed by THF (7 × 800 μL). The support was then suspended in a freshly prepared solution of THF (1 mL) containing acetic anhydride (10% v/v), 2,6-lutidine (11% v/v), and *N*-methylimidazole (16% v/v) and shaken for 15 min at room temperature. The support was then washed with THF (2 × 800 μL), and the treatment with acetic anhydride, 2,6-lutidine and *N*-methylimidazole was repeated. The support was washed with THF (7 × 800 μL), followed by methanol (7 × 800 μL), and then dried under vacuum at room temperature for 4 h to yield **6a**, **6b**, **9a** and **9b**. Fmoc-SU-8 (less than 200 mg) was suspended in piperidine (20% in DMF, 1 mL) and shaken at room temperature for 20 min. The treatment with piperidine/DMF was repeated. The support was washed with DMF (10 × 800 μL), followed by tetrahydrofuran (THF) (8 × 800 μL) and diethyl ether (3 × 800 μL), and dried under vacuum at room temperature for 4 h to yield **7a**, **7b**, **10a**, and **10b**.

Attachment of HMPA linker (Synthesis of 11a and 11b). HMPA (8 mg, 45 μmol) was dissolved in DMF (100 μL), and DIC (7 μL, 45 μmol) was added. The mixture was shaken for 8 min at room temperature (RT); HOBt (6 mg, 45 μmol) was added, and the mixture was shaken for 5 min at room temperature. The mixture was added to amino SU-8 (50 mg, 850 nmol based on free $-NH_2$ groups) suspended in DMF (100 μL), and the mixture was shaken for 1 h at room temperature. The support was washed with DMF (7 × 800 μL), and the procedure was repeated. The support was washed with DMF (7 × 800 μL), followed by methanol (7 × 800 μL), and dried under vacuum at room temperature for 4 h.

Attachment of First Amino Acid. *N*-α-Fmoc-Leucine (12 mg, 33 μmol) was dissolved in DMF (50 μL), and DIC (5 μL, 33 μmol) was added. The mixture was shaken for 8 min at RT. DMAP (0.5 mg, 3 μmol) was added, and the mixture was added to HMPA SU-8 (850 nmol based on loss of amino groups after the attachment of the linker) suspended in DMF (100 μL); then, the mixture was shaken for 1 h at room temperature. The support was washed with DMF (7 × 800 μL), and the procedure was repeated twice. The support was washed with DMF (7 × 800 μL), followed by methanol (7 × 800 μL), and dried under vacuum at room temperature for 4 h.

Peptide Synthesis. The following amino acids were required: *N*-α-Fmoc-Leu, *N*-α-Fmoc-Gly, *N*-α-Fmoc-Phe, *N*-α-Fmoc-Val, *N*-α-Fmoc-Gln, *N*-α-Fmoc-Asn, *N*-α-Fmoc-Pro, *N*-α-Fmoc-Ile, *N*-α-Fmoc-(*O*-Trt)-Ser, and *N*-α-Fmoc-(*O*-2-Cl-Trt)-Tyr. *N*-α-Fmoc-amino acid (8 μmol) was dissolved in DMF (50 μL), and TBTU (3 mg, 9 μmol), HOBt (0.3 mg) and *N*,*N*-diisopropyl-*N*-ethylamine (DIPEA) (1.5 μL, 9 μmol) were added. The mixture was shaken for 2 min; then the mixture was added to the deprotected SU-8 (850 nmol based on $-NH_2$ groups) suspended in DMF (100 μL), and the mixture was shaken for 1 h at RT. The support was washed with DMF (3 × 800 μL), followed by methanol (2 × 800 μL) and diethyl ether (3 × 800 μL). The completeness of the reaction was monitored by the ninhydrin test. After a negative ninhydrin test, the *N*-terminal Fmoc group was removed. The peptide was cleaved from the support by treatment with TFA/phenol (98/2% v/w, 25 mL/g of resin) for 90 min at room temperature. The support was filtered and washed with TFA (3 × 1 mL). The combined filtrates were evaporated under vacuum, and the residual oil was triturated with diethyl ether. The solid that precipitated was washed with diethyl ether and dried under vacuum at room temperature for 4 h.

LC-MS Analysis of Peptides. Dry samples were dissolved in acetonitrile/H_2O (50/50% v/v) (0.5 mg/mL). Analytical HPLC (Gilson) was monitored at 215 and 280 nm using a Phenomenex C18 column (150 × 4.5 mm, 5 μm, 300 Å pore size). The gradient was 10−100% B in A gradient over 32 min at 1 mL/min, where A was 0.1% TFA /10% acetonitrile/water and B was 0.1% TFA/acetonitrile. The injection volume was 200 μL. ESI-MS (Surveyor MSQ) was coupled online with the HPLC separation by a splitter (1/4 split) and mixing the chromatographic outlet with 0.3% formic acid/50% acetonitrile/50% H_2O in a third pump (1 mL/min). See main text for results.

HIV Protease I substrate, **13**, was further characterized by NMR spectroscopy using a Bruker spectrometer operating at either 400 MHz (^1H) or 100 MHz (^{13}C). ^1H NMR (D_2O): δ 7.06 (m, 2H, J = 8.7 Hz), 6.78 (d, 1H, J = 8.6 Hz), 6.75 (d, 1H, J = 8.2 Hz), 4.74 (dd, 1H, J = 8.6, 5.6 Hz), 4.58 (dd, 1H, J = 7.9, 6.0 Hz), 4.45 (t, 1H, J = 6 Hz), 4.36 (dd, 1H, J = 7.0, 6.4 Hz), 4.31 (dd, 1H, J = 9.0, 5.0 Hz), 4.24 (dd, 1H, J = 8.4, 5.6 Hz), 4.11−4.03 (m, 2H), 3.79 (m, 3H), 3.67 (dd, 1H, J = 7.1, 16.5 Hz), 3.48 (dd, 1H, J = 6.4, 16.0 Hz), 3.01 (dd, 1H, J = 5.0, 14.6 Hz), 2.79 (m, 1H), 2.68 (m, 1H), 2.56 (m, 2H), 2.29 (m, 3H), 2.17 (m, 4H), 2.00−1.76 (m, 7H), 1.44 (m, 1H), 1.13 (m, 1H), 0.95 (m, 6H), 0.89−0.78 (m, 12 H). ^{13}C NMR (D_2O): δ 178.0, 174.9, 174.6, 174.1, 173.9, 173.5, 172.1, 171.8, 171.5, 171.4, 169.8, 154.8, 131.1, 130.9, 128.4, 61.4, 60.7, 59.7, 58.8, 58.7, 55.8, 53.4, 53.3, 52.4, 50.6, 48.3, 36.6, 36.4, 35.9, 31.5, 31.3, 30.6, 30.3, 29.6, 27.2, 26.8, 25.1, 25.0, 18.7, 18.3, 18.2, 18.0, 17.1, 15.2, 10.5

Synthesis of 17. 3′-*O*-Succinimidyl-5′-*O*-4,4′-dimethoxytrityl-*N*-benzoyl-2′-deoxycytidine (8.0 mg, 11 μmol) was dissolved in DMF (500 μL). HOBt (0.3 mg, 2 μmol), TBTU

(3.5 mg, 11 μmol), and DIPEA (1.8 μL, 11 μmol) were added, and the mixture was stirred at room temperature for 5 min. The mixture was added to amino SU-8 **10** (200 mg, 3.5 μmol based on $-NH_2$ groups) and stirred vigorously at room temperature for 30 min. The support was washed with DMF (3 × 800 μL), and the procedure was repeated. The support was washed with DMF (7 × 800 μL), followed by THF (7 × 800 μL), and dried under vacuum at room temperature for 4 h.

Oligonucleotide Synthesis, Purification, and Analysis.[36,39] A MerMade 192 automatic synthesizer (Bioautomation Inc.) was used for oligonucleotide synthesis according to the manufacturer protocol for a 50 nmol scale synthesis optimized for MerMade CPG columns (standard CPG loaded with 3′-C, 110 μm nominal particle size, 1000 Å pore size, loading 20−60 μmol/g). All reagents were obtained from Link Technologies, Bellshill, Scotland. For SU-8 particles, a modified protocol was used involving phosphoramidite double couplings. After the synthesis, the oligonucleotides were cleaved from the support using ammonia solution (35%, 0.88 g/mL, 1 × 150 μL for 15 min, followed by 3 × 100 μL for 15 min, filtration, and collection of the filtrate each time). The combined filtrates were heated in a sealed plate at 65 °C for 6 h. The solutions were freeze-dried overnight, and the residue was dissolved in H_2O (150 μL). The oligonucleotide solutions were purified by HPLC. Preparative HPLC (Gilson) was monitored at 254 and 280 nm. Separation was carried out using a Phenomenex Jupiter C18 column (50 × 4.60 mm, 5 μm, 300 Å pore size). The gradient was 0−40% B in A gradient over 8 min, isocratic at 40% B in A for 30 s, then 40−0% B in A in 30 s, and isocratic at 100% A for 1 min, at 1 mL/min, where A was 0.1 M ammonium acetate pH 7 in water and B was 0.1 M ammonium acetate pH 7 in 50% acetonitrile in water. The injection volume was 120 μL. Isolated peak detection allowed for collection of single peak fractions between 2 and 7 min. After the fractions were freeze dried, they were analyzed by capillary electrophoresis using a Beckman-Coulter P/ACE MDQ capillary electrophoresis system following the manufacturer's protocol using a Beckman eCAP ssDNA 100-R kit. Separation was carried out in tris-borate urea (7 M) buffer using an eCAP DNA 100 μm ID capillary (20 cm). Fresh eCAP ssDNA gel was loaded into the capillary under pressure (60 psi × 15 min), followed by equilibration of the capillary immersed in tris-borate urea (7 M) buffer at constant voltage (3 kV, 0.17 min ramp, normal polarity × 5 min, followed by 9 kV, 0.17 min ramp, normal polarity × 10 min). The capillary temperature was set at a constant 30 °C. The sample (diluted to 0.5 OD at 254 nm) was loaded at constant voltage (10 kV × 2 s, reverse polarity) and then was separated at constant voltage (9 kV × 60 min, 0.17 min ramp, reverse polarity). UV monitoring was carried out at 254 nm. Migration times were compared to a standard oligonucleotide ladder (Beckman-Coulter) and confirmed by comigration of samples. Oligonucleotides were further characterized using a Dynamo MS MALDI-TOF spectrometer as described elsewhere.[36] Samples of T_{10}, T_{15}, and T_{20} were used as internal standards.

Acknowledgment. This project was supported by Research Councils, U.K., through the Basic Technology Programme. P.L.R. thanks the Royal Society for support via a University Research Fellowship.

Supporting Information Available. MS and NMR of peptides. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Shaw, J. M.; Gelorme, J. D.; LaBianca, N. C.; Conley, W. E.; Holmes, S. J. *IBM J. Res. Dev.* **1997**, *41*, 81−94.
(2) Lorenz, H.; Despont, M.; Fahrni, N.; Brugger, J.; Vettiger, P.; Renaud, P. *Sens. Actuators, A* **1998**, *64*, 33−39.
(3) Lorenz, H.; Despont, M.; Fahrni, N.; LaBianca, N.; Renaud, P.; Vettiger, P. *J. Micromech. Microeng.* **1997**, *7*, 121−124.
(4) Ribeiro, J. C.; Minas, G.; Turmezei, P.; Wolffenbuttel, R. F.; Correia, J. H. *Sens. Actuators, A* **2005**, *123−24*, 77−81.
(5) Sikanen, T.; Tuomikoski, S.; Ketola, R. A.; Kostiainen, R.; Franssila, S.; Kotiaho, T. *Lab Chip* **2005**, *5*, 888−896.
(6) Koerner, T.; Brown, L.; Xie, R. X.; Oleschuk, R. D. *Sens. Actuators, B* **2005**, *107*, 632−639.
(7) Schneider, A.; Su, B.; Button, T. W.; Singleton, L.; Wilhelmi, O.; Huq, S. E.; Prewett, P. D.; Lawes, R. A. *Microsyst. Technol.* **2002**, *8*, 88−92.
(8) Lee, J.; Shin, H.; Kim, S.; Hong, S.; Chung, J.; Park, H.; Moon, J. *Jpn. J. Appl. Phys., Part 2* **2003**, *42*, L1171−L1174.
(9) Genolet, G.; Despont, M.; Vettiger, P.; Anselmetti, D.; de Rooij, N. F. *J. Vac. Sci. Technol., B* **2000**, *18*, 617−620.
(10) Chang-Yen, D. A.; Gale, B. K. *Lab Chip* **2003**, *3*, 297−301.
(11) Evans, M.; Sewter, C.; Hill, E. *Assay Drug Dev. Technol.* **2003**, *1*, 199−207.
(12) Kaye, P. H.; Tracey, M. C. (University of Hertfordshire, UK). Br. Patent GB96-22249 2306484, 1997.
(13) Preininger, C.; Sauer, U. *Sens. Actuators, B* **2003**, *90*, 98−103.
(14) Preininger, C.; Bodrossy, L.; Sauer, U.; Pichler, R.; Weilharter, A. *Anal. Biochem.* **2004**, *330*, 29−36.
(15) Preininger, C.; Sauer, U.; Dayteg, J.; Pichler, R. *Bioelectrochemistry* **2005**, *67*, 155−162.
(16) Marie, R.; Schmid, S.; Johansson, A.; Ejsing, L.; Nordstrom, M.; Hafliger, D.; Christensen, C. B. V.; Boisen, A.; Dufva, M. *Biosens. Bioelectron.* **2006**, *21*, 1327−1332.
(17) Song, I. H.; Ajmera, P. K. *J. Micromech. Microeng.* **2003**, *13*, 816−821.
(18) Nordstrom, M.; Johansson, A.; Nogueron, E. S.; Clausen, B.; Calleja, M.; Boisen, A. *Microelectron. Eng.* **2005**, *78−79*, 152−157.
(19) Carlier, J.; Arscott, S.; Thomy, V.; Fourrier, J. C.; Caron, F.; Camart, J. C.; Druon, C.; Tabourier, P. *J. Micromech. Microeng.* **2004**, *14*, 619−624.
(20) Jackman, R. J.; Floyd, T. M.; Ghodssi, R.; Schmidt, M. A.; Jensen, K. F. *J. Micromech. Microeng.* **2001**, *11*, 263−269.
(21) Nastasovic, A.; Jovanovic, S.; Dordevic, D.; Onjia, A.; Jakovljevic, D.; Novakovic, T. *React. Funct. Polym.* **2004**, *58*, 139−147.
(22) Bayramoglu, G.; Kaya, B.; Yakup Arica, M. *Food Chem.* **2005**, *92*, 261−268.
(23) Wellings, D. A.; Atherton, E. *Methods Enzymol.* **1997**, *289*, 44−67.
(24) Merrifield, B. *Methods Enzymol.* **1997**, *289*, 3−13.
(25) Shin, D. S.; Kim, D. H.; Chung, W. J.; Lee, Y. S. *J. Biochem. Mol. Biol.* **2005**, *38*, 517−525.
(26) Miao, W.; Chan, T.-H. *J. Org. Chem.* **2005**, *70*, 3251−3255.
(27) Mizuno, M.; Goto, K.; Miura, T.; Matsuura, T.; Inazu, T. *Tetrahedron Lett.* **2004**, *45*, 3425−3428.
(28) Boas, U.; Christensen, J. B.; Jensen, K. J. *J. Comb. Chem.* **2004**, *6*, 497−503.

(29) Kiso, Y. *Biopolymers* **1996**, *40*, 235−44.
(30) De Mendoza, C.; Soriano, V. *Curr. Drug Metab.* **2004**, *5*, 321−328.
(31) Jiang, T.; Olson, E. S.; Nguyen, Q. T.; Roy, M.; Jennings, P. A.; Tsien, R. Y. *Proc. Nat. Acad. Sci. U.S.A.* **2004**, *101*, 17867−17872.
(32) Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1992**, *48*, 2223−311.
(33) Caruthers, M. H.; Barone, A. D.; Beaucage, S. L.; Dodds, D. R.; Fisher, E. F.; McBride, L. J.; Matteucci, M.; Stabinsky, Z.; Tang, J. Y. *Methods Enzymol.* **1987**, *154*, 287−313.
(34) Hu, S.; Dovichi, N. J. *Anal. Chem.* **2002**, *74*, 2833−2850.
(35) Righetti, P. G.; Gelfi, C. *J. Biochem. Biophys. Methods* **1999**, *41*, 75−90.
(36) Langley, G. J.; Herniman, J. M.; Davies, N. L.; Brown, T. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 1717−1723.
(37) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147−157.
(38) Newcomb, W. S.; Deegan, T. L.; Miller, W.; Porco, J. A. *Biotechnol. Bioeng.* **1998**, *61*, 55−60.
(39) Brown, T.; Brown, D. J. S. *Methods Enzymol.* **1992**, *211*, 20−35.

CC060079P