Cleavage and Analysis of Material from Single Resin Beads

Neil J. Wells, Michael Davies, and Mark Bradley*

Department of Chemistry, University of Southampton, Southampton SO17 1BJ, U.K.

Received March 3, 1998

The screening of individual compounds in solution, cleaved from single resin beads,[1] has considerable but as yet unrealized potential in the search for new therapeutic agents. Single-bead screening allows the effective application of split and mix methodology,[2] which potentially enables huge numbers of compounds to be prepared in an extremely economical fashion. A limitation of this methodology at the present time is the limited quantity of material on a single bead for routine and repeated screens.[3] For this technology to become generally applicable, we believe that bead loading must be increased to a level where single beads have the capacity to release sufficient material for multiple screens and ideally HPLC analysis and compound purification. The resin materials for these studies need to be inexpensive, physically robust, and compatible with a wide range of chemistries;[4] they must also be monodispersive or at least available with a narrow size distribution. In addition, the number of beads per gram must be practical for library synthesis.[5]

Recently, we reported the solid-phase synthesis of PAM-AM (polyamidoamine)-type dendrimers on TentaGel resin[6] as a means of both enhancing bead loading and as an efficient method of dendrimer synthesis.[7] This also represents a method of multiple ligand presentation for the screening of weakly binding ligands, an area that has received increased attention recently.[8] In this paper, we report the preparation of high-loading resin beads such that a single bead gives sufficient material for NMR characterization, mass spectrometry, and repeated conventional HPLC analysis. The high-loading beads were observed to be exceptionally robust, compatible with a wide range of solvents, stable to prolonged storage, and easy to handle for both synthesis and single-bead manipulations. Thus, chloromethylated PS resin beads (Polymer Laboratories, 250−300 μm, 2 mmol g^{-1}) were converted to aminomethyl resin using either potassium phthalimide[9] or potassium bis(*tert*-butylimino)dicarbonate[10] to give, after hydrazinolysis and acidolysis respectively, aminomethyl resin with a measured amine loading of 1.03 mmol g^{-1} (Fmoc[11] and ninhydrin[12] tests). A small inert PEG spacer was then introduced[13] to allow efficient PAMAM dendrimer synthesis. The use of the acid-labile Wang-type linker allowed the dendrimer to be fully characterized and was essentially homogeneous as judged by ESMS and NMR.[14] The beads proved to be physically robust, surviving dendrimer synthesis which arbitrarily used a 2 day per half-generation reaction time at 50 °C.

To test the synthetic utility of these high-loading PS-PEG-[G 2.0] beads (polystyrene-poly(ethyleneglycol)-dendrimer generation 2.0), the peptide Fmoc-Val-Phe-Ala-OH was prepared using standard peptide coupling conditions on the HMPB linker.[15] This linker allows the compound to be cleaved from the solid support with 1% TFA in CH_2Cl_2, leaving the dendrimer−resin link intact. The data for this peptide, when cleaved from a single resin bead (i.e., all the data shown are from the same bead), are shown in Figure 1. Figure 1a shows the 500 MHz ^{1}H NMR data for the peptide, following in situ cleavage in the NMR tube. This was achieved by placing a single resin bead supporting the peptide Fmoc-Val-Phe-Ala-OH into a coaxial NMR tube with 1% F_3CCO_2D (99.5%D) in $CDCl_3$ (99.96%D) and the NMR spectrum being recorded with the residual F_3CCO_2H resonance suppressed. The resonances for the peptide are clearly visible and identical to a synthesized standard (Figure 1b). A portion (25%) of this NMR sample was then analyzed by reversed-phase HPLC on a 150 × 3.0 mm Prodigy C_{18} column and gave the data shown in Figure 1c. A portion of the sample was also diluted and analyzed by ESMS and gave the data shown in Figure 1d. The material obtained from a single bead was determined to be approximately 32 nmol by internal HPLC calibration with a presynthesized standard and demonstrates that the overall synthetic efficiency, which includes dendrimerization, peptide synthesis, single-bead cleavage, and HPLC manipulation was very high.

There were approximately 34 500 beads/g in the dendrimer derivatized form, which is acceptable for split and mix library applications.

A small library was synthesized based on Leu-enkephalin-Lys[15] (Tyr-Gly-Gly-Phe-Leu-Lys). Twenty compounds of the general structure Xaa_{1-20}-Gly-Gly-Phe-Leu-Lys were prepared and analyzed by HPLC and ESMS. Figure 2 shows these data for two library members, following single-bead cleavages. The desired compounds were in all cases the major product. Impurities, predominantly due to incomplete side-chain deprotection following the relatively mild 1% TFA

(1) (a) Salmon, S. E.; Lam, K. S.; Lebl, M.; Kandola, A.; Khattri, P. S.; Wade, S.; Pátek, M.; Kocis, P.; Krchnák, V.; Felder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 11708−11712. (b) Burbaum, J. J.; Ohlmeyer, M. H. J.; Reader, J. C.; Henderson, I.; Dillard, L. W.; Li, G.; Randle, T. L.; Sigal, N. H.; Chelsky, D.; Baldwin, J. J. *Ibid.* **1995**, *92*, 6027−6031.

(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) A single polystyrene resin bead with a diameter of 100 μm and a substitution of 1.05 mmol g^{-1} will have approximately 500 pmol of sites. A single Tentagel-S-NH_2 resin bead (130 μm) with a substitution of 0.3 mmol g^{-1} has approximately 350 pmol of sites.

(4) In our experience, some beaded materials are not mechanically stable enough to withstand the manipulations and repeated solvation and drying associated with a number of synthetic steps, a factor especially true with some of the larger grafted beads.

(5) With ultralarge 750 μm diameter beads supplied by Rapp Polymere GmbH there are around 4600 beads/g. Rapp, W. In *Combinatorial Chemistry Synthesis and Applications*; Wilson, S. R., Czarnik, A. W., Eds.; Wiley: New York, Chichester, Weinheim, Brisbane, Singapore, Toronto, 1997; Vol. 4, pp 65−93.

(6) Swali, V.; Wells, N. J.; Langley, G. J.; Bradley, M. *J. Org. Chem.* **1997**, *62*, 4902−4903.

(7) Tomalia, D. A.; Naylor, A. M.; Goddard, W. A., III. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 138−175.

(8) (a) Linares, M.; Roy, R. *J. Chem. Soc., Chem. Commun.* **1997**, 2119−2120. (b) Hansen, H. C.; Haataja, S.; Finne, J.; Magnusson, G. *J. Am. Chem. Soc.* **1997**, *119*, 6974−6979. (c) Zanini, D.; Roy, R. *J. Org. Chem.* **1996**, *61*, 7348−7354.

(9) Mitchell, A. R.; Erickson, B. W.; Ryabtsev, M. N.; Hodges, R. S.; Merrifield, R. B.; *J. Am. Chem. Soc.* **1976**, *98*, 7357−7362.

(10) (a) Creba, J.; Ragnarsson, U. *Synthesis* **1987**, 275−276. (b) Allan, R. D.; Johnston, G. A. R.; Kazlauskas, R.; Tran, H. W. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2983−2985.

(11) Atherton, E.; Sheppard, R. C. *Solid-Phase Peptide Synthesis*: *A Practical Approach*; IRL Oxford Univ. Press: Oxford, 1989.

(12) Sarin, V. K.; Kent, S. B. H.; Tam, J. P.; Merrifield, R. B. *Anal. Biochem.* **1981**, *117*, 147−157.

(13) Cook, R. M.; Adams, J. H.; Hudson, D. *Tetrahedron Lett.* **1994**, *35*, 6777−6780.

(14) ^{1}H NMR (300 MHz, D_2O): δ 3.64−3.51 (m, 16H); 3.49−3.33 (br m, 40H); 3.31−3.21 (m, 12H); 3.10−3.04 (m, 16H); 2.80−2.71 (br m, 20H); 2.20−2.05 (br m, 4H). ^{13}C NMR (75 MHz, D_2O): δ 175.2 (*C*O); 174.6 (*C*O); 54.1 (2); 52.3 (2); 51.7 (2); 46.1 (2); 41.5 (2); 39.4 (2); 36.7 (2); 31.4 (2), 31.1 (2); 23.1 (2). MS ES+: 301.3 (100) M + $5H^{5+}$.

(15) (a) Riniker, B.; Florsheimer, A.; Fretz, H.; Sieber, P.; Kamber, P. B. *Tetrahedron* **1993**, *49*, 9307−9320. (b) Florsheimer, A.; Riniker, B. In *Peptides 1990*; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden 1991, 131−133.

S0022-3263(98)00394-6 CCC: $15.00 © 1998 American Chemical Society
Published on Web 08/25/1998

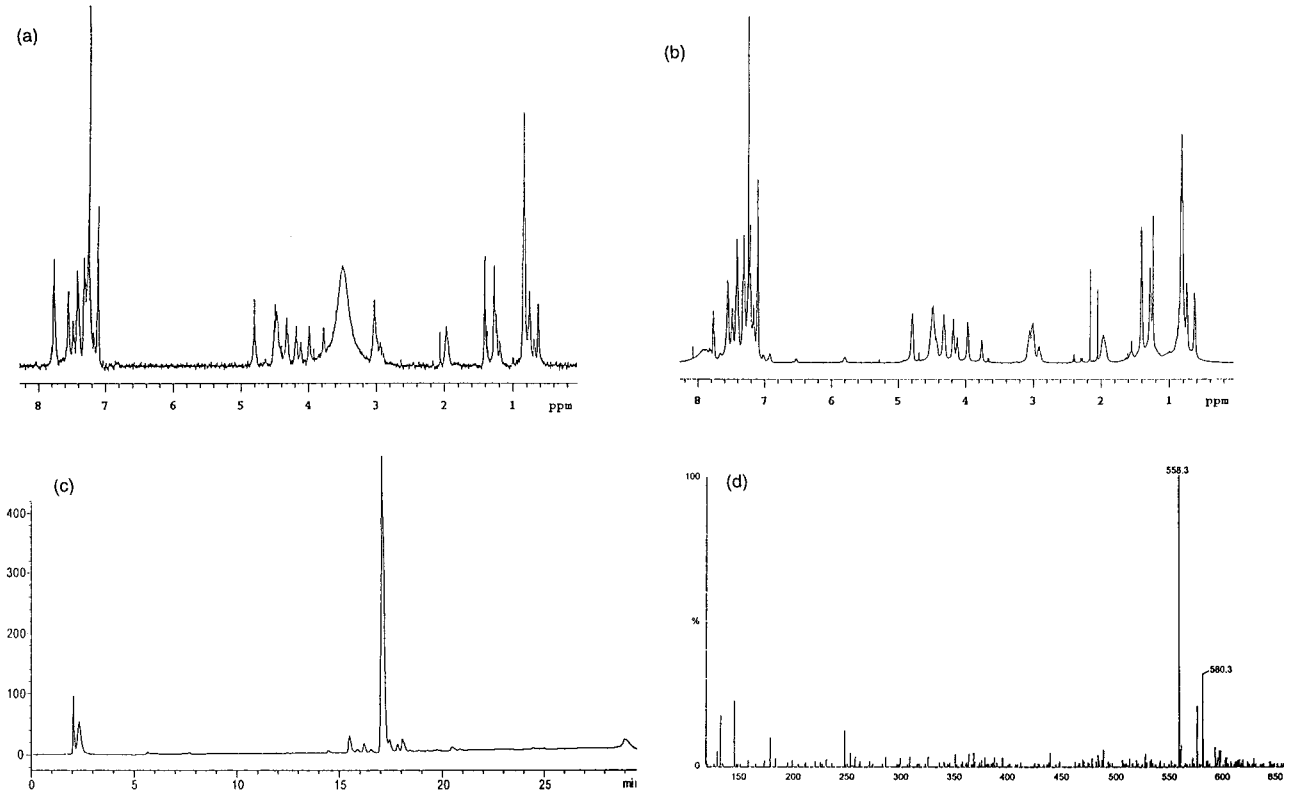

Figure 1. (a) In situ cleavage and analysis of Fmoc-Val-Phe-Ala-OH. (b) NMR of Fmoc-Val-Phe-Ala-OH synthesized independently. (c) 25% of the NMR sample analyzed by reversed-phase HPLC 150 mm × 3.0 mm Prodigy C_{18} column, $\lambda = 270$ nm. (d) 1% of NMR sample analyzed by ESMS.

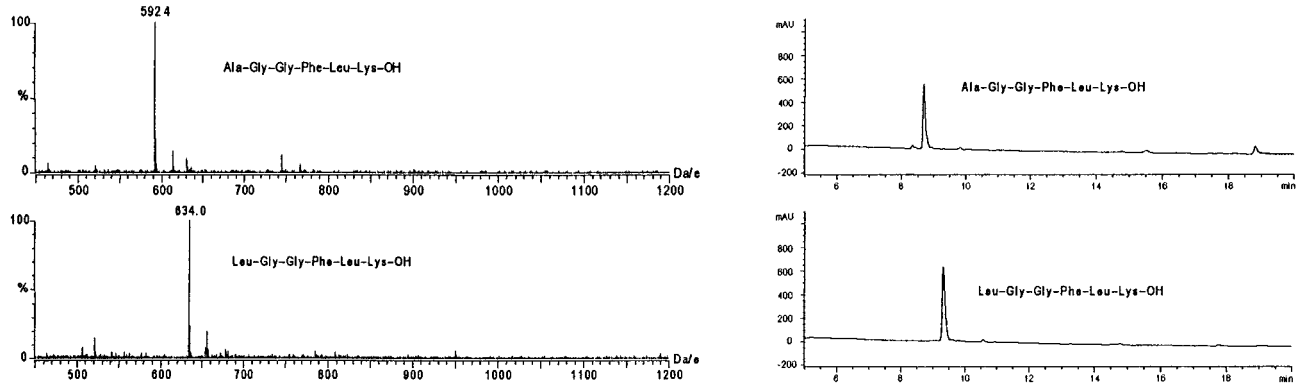

Figure 2. HPLC and ESMS data of two members of the Leu-enkephalin-Lys library ($\lambda = 220$ nm, 150 mm × 3.0 mm Prodigy C_{18} column).

in CH_2Cl_2 cleavage of the peptide from the HMPB linker, were observed, but this was only found to be particularly significant in the case of the Arg(Pmc)[17] containing peptide.

In conclusion, we have produced resin beads that are physically robust and have a very high loading. We have shown that a single bead affords enough material for comprehensive analysis and demonstrated their synthetic utility in the preparation of a small peptide library. Interestingly, although initial reactions on the large PS beads appeared slow, the dendrimer derivatized materials reacted rapidly and required nonforcing conditions for peptide synthesis.

Acknowledgment. We thank the Royal Society for a University Research Fellowship (M.B.), the EPSRC for a research grant (N.J.W.), the BBSRC (BMS, ROPA grant), and Dr. David Turner of the University of Southampton for assistance with the high-field NMR experiments.

Supporting Information Available: Experimental details for the synthesis of the peptide library, NMR data for the library members shown in the text, and ESMS and HPLC data for 20 library members (18 pages).

JO9803941

(16) Vicar, J.; Servitova, L.; Flegel, M.; Hauzer, K.; Barth, T. *Collect. Czech. Chem. Commun.* **1985**, *50*, 1329−1334.

(17) (a) Green, J.; Ogunjobi, O. M.; Ramage, R.; Stewart, A. S. J. *Tetrahedron Lett.* **1988**, *29*, 4341−4344. (b) Ramage, R.; Green, J. *Ibid.* **1987**, *28*, 2287−2290.