



Pergamon

SCIENCE @ DIRECT®

Tetrahedron Letters 44 (2003) 1751–1754

TETRAHEDRON
LETTERS

Saturated resins or stress of the resin

Glòria Sanclimens,^{a,b} Laia Crespo,^b Miquel Pons,^b Ernest Giralt,^{a,b} Fernando Albericio^{a,b,*} and Miriam Royo^{b,c,*}

^aBarcelona Biomedical Research Institute, Barcelona Science Park, University of Barcelona, Josep Samitier 1, 08028 Barcelona, Spain

^bDepartment of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain

^cCombinatorial Chemistry Unit, Barcelona Science Park, University of Barcelona, Josep Samitier 1, 08028 Barcelona, Spain

Received 10 January 2003; accepted 13 January 2003

Abstract—Although the solid-phase mode is an excellent strategy for the preparation of both biomolecules and small molecules, the synthesis of polyproline-based dendrimers has provided evidence that the capacity of the bead is limited. This phenomenon, which can be interpreted as *saturation* or *stress* of the resin, can lead to a complete breakdown of the bead structure. © 2003 Elsevier Science Ltd. All rights reserved.

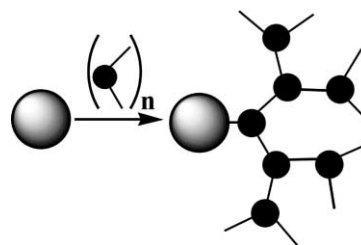
1. Introduction

Modern Chemistry owes a great debt to R. Bruce Merrifield for the establishment of the solid-phase method for the synthesis of peptides.¹ In the sixties, seventies, and most of the eighties, the solid-phase approach was mainly applied to the synthesis of biomolecules such as peptides,² oligonucleotides,³ oligosaccharides,⁴ and related non-natural polymers [oligocarbamates,⁵ peptide nucleic acids (PNA)⁶ etc.]. At the beginning of the nineties the publication of the seminal paper by Jon Ellman, which described the solid-phase synthesis of a benzodiazepine library,^{7,8} can be considered as the start of a new era in synthetic chemistry.⁹ More recently, solid-phase strategies have also been used for the synthesis of more complex polymers such as dendrimers.¹⁰ Thus, it is possible to affirm that Merrifield's prediction: "...it seems quite clear that a gold mine is awaiting the organic chemist who would look to solid supports for controlling and directing his synthetic reactions"¹¹ is now a reality.

The most significant difference between the solid and solution phases is the presence of the solid support in the former case.¹² The success of a particular synthetic

process is therefore strongly related to the choice of support and its performance. What is the role of the solid support in the synthetic process? While the *handle* or *linker* should be thought of as a protecting group,¹³ the solid support should be considered as being analogous to a co-solvent.¹⁴ For example, when a reaction is carried out on a polystyrene solid support, it can be considered that toluene is a co-solvent in the reaction.

One of the problems associated with the solid-phase is the degradation and even the destruction of the bead. The reasons reported for this phenomenon are: (i) lack of stability to acidic, basic, reducing and oxidizing conditions used in the reactions; (ii) mechanical erosion of the bead, e.g., energetic stirring; (iii) the beads are not sufficiently stable to withstand the repeated solvation and drying associated with a large number of synthetic steps.



Scheme 1. Solid-phase strategy for the preparation of proline-based dendrimers.

Keywords: beads; dendrimers; globular macromolecules; solid-phase; solid support.

* Corresponding authors. Tel.: +00 34 93 403 70 88; fax: +00 34 93 403 71 26 (F.A.); tel.: +00 34 93 403 71 22; fax: +00 34 93 403 71 26 (M.R.); e-mail: albericio@pcb.ub.es; mroyo@pcb.ub.es

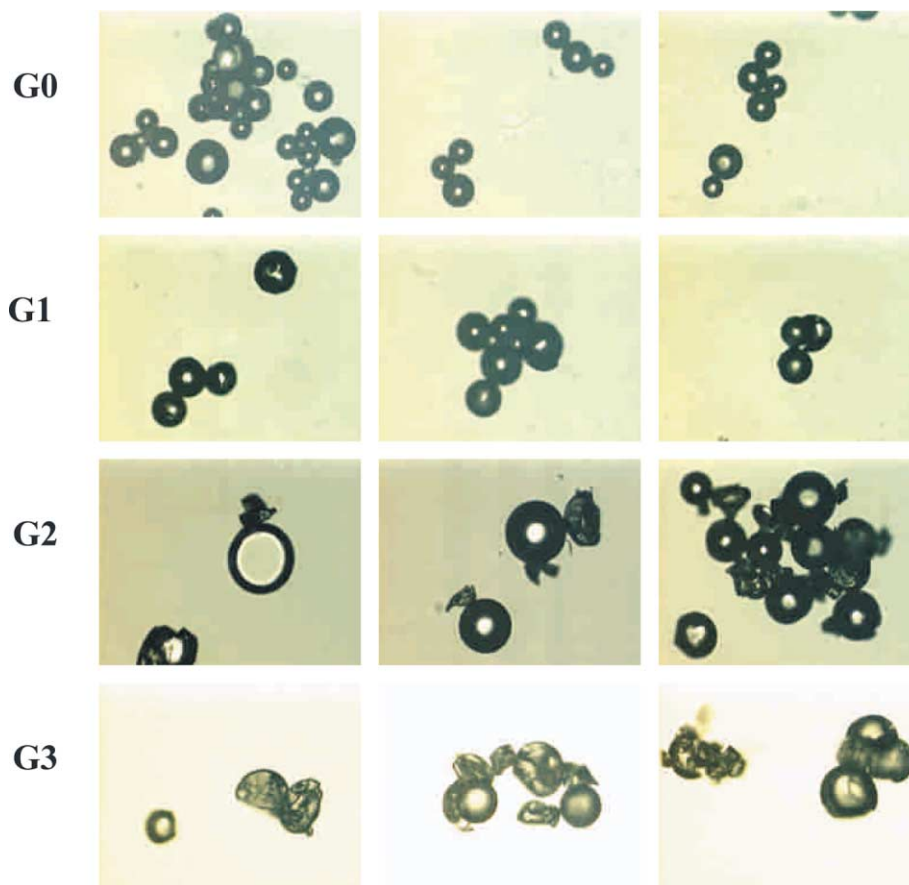


Figure 1. Photomicrographs of representative beads at different generation stages.

We describe here another possible cause of bead degradation, which is related to the mass/volume ratio of the compound anchored on the bead. This phenomenon can be related with the concept of saturated solutions. In contrast to the situation in saturated solutions, where the solute precipitates, in a *saturated solid support* the complete breakdown of the bead occurs. It is proposed that this phenomenon can also be called *stress of the bead*.

2. Results and discussion

Polyproline-based dendrimers were synthesized following an orthogonal convergent (reversed approach, from *in to out*)¹⁵ strategy—as described previously (Scheme 1)^{16,17}—on a *p*-methylbenzhydrylamine (MBHA) resin (loading 0.40 mmol/g).

Dendrons (●) of 13 residues (two branches of 6 residues each) built on an imidazoline branching unit were sequentially incorporated onto MBHA resin. This strategy involves very few synthetic steps on the resin (deprotection and incorporation of each dendron) and therefore precludes the occurrence of the phenomena described above that typically give rise to solid support degradation. All resin beads containing different generations of dendrimers were swollen in CH₂Cl₂ to assess their physical appearance by optical microscopy.

Figure 1 shows representative photomicrographs of beads at different generation stages. There is evidence of some deterioration of beads even at G2. This process is more marked in G3 dendrimers, where some of the beads have completely burst.

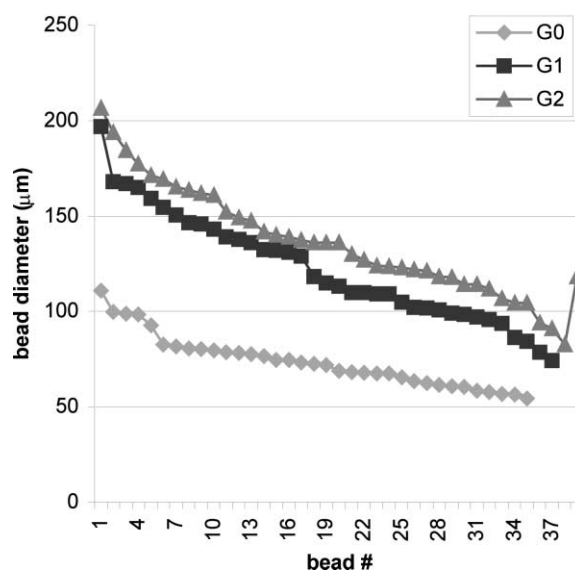


Figure 2. Correlation between average diameter (μm) versus generation.

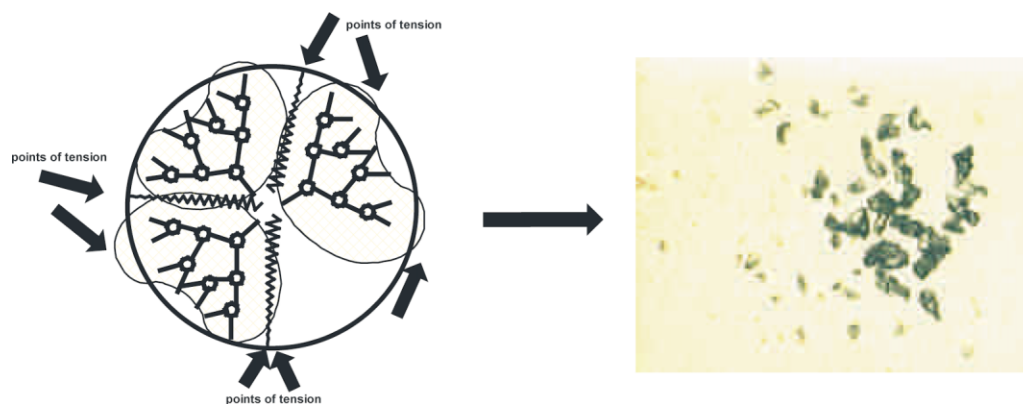


Figure 3. Sketch of the tension suffered by the beads and photomicrograph of totally destroyed bead(s).

Figure 2 shows the correlation between average diameter (μm) and generation (approximately 40 beads were analyzed for each generation). Taking into account these data, the volume (V_1) of the bead at G1 (generation 1) is clearly superior (more than 4 times) to V_0 . However, this huge increase in the volume is not associated with the transition from G1 to G2. Indeed, V_2 is 32% larger than V_1 but V_3 , determined from those beads that had not burst, shows only a moderate increase when compared with V_2 . This situation provides more evidence for the idea of resin saturation.

Similar results were also observed when a hydroxymethyl (Merrifield) resin of lower loading (0.23 mmol/g) was used.

Bradley and co-workers described briefly a similar phenomenon when high-loading resins using a dendrimer approach were prepared.^{18–20} These researchers describe how their larger beads are not as robust as the smaller beads due to two factors: (i) a solvent shock that causes the beads to shatter, necessitating a gradual change from one solvent to another and gentle handling;²⁰ and (ii) the rapid formation of CO_2 within the bead, which then diffuses through the polystyrene matrix, during the removal of the *tert*-butoxycarbonyl (Boc) group with trifluoroacetic acid (TFA).¹⁹ Although these two factors could partially contribute to the phenomenon in question, they do not apply in our case because just one solvent, DMF, was used in all processes and TFA was not used at all.

3. Conclusions

The solid-phase preparation of polyproline-based dendrimers has provided evidence that the capacity of the bead is limited. High ratios of dendrimer mass to bead volume could lead to a situation where most of the beads experience enormous tension. These points of tension evolve and give rise to *stress* in the bead, which eventually bursts (Fig. 3). This process can also be envisaged in terms of a *saturated resin*, in analogy to a saturated solution. Therefore, special care should be taken when the solid-phase is used for the preparation of this kind of globular macromolecule.

3.1. Experimental protocols

Solid-phase synthesis of the dendrimers was carried out as described previously on an MBHA resin (0.40 mmol/g, after partial incorporation of a glycine residue to reduce the initial loading of 0.68 mmol/g provided by the supplier, Novabiochem AG., L  ufelfingen, Switzerland).^{16,17} Optical microscopy was carried out using an Olympus BH2-UMA (100 \times).

Acknowledgements

M.R. is a Ramon y Cajal fellow (MCyT, Spain). The excellent technical assistance of Ms. Marisol Cu  arro (Technical Scientific Services, University of Barcelona) warrants special acknowledgement. This work was partially supported by CICYT (BQU2000-0235, BQU2002-02047, and BIO2002-02301), Generalitat de Catalunya [Grup Consolidat, and Centre de Refer  ncia en Biotecnologia], and Fundaci   La Marat  -TV3.

References

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
2. Lloyd-Williams, P.; Albericio, F.; Giralt, E. *Chemical Approaches to the Synthesis of Peptides and Proteins*; CRC Press: Boca Raton FL, 1997.
3. Eritja, R. In *Solid-Phase Synthesis. A Practical Guide*; Kates, S. A.; Albericio, F., Eds.; Marcel Dekker, Inc: New York, 2000; pp. 529–548.
4. Roberge, J. Y. In *Solid-Phase Synthesis. A Practical Guide*; Kates, S. A.; Albericio, F., Eds.; Marcel Dekker, Inc: New York, 2000; pp. 549–615.
5. Cho, C. Y.; Moran, E. J.; Cherry, S. R.; Stephans, J. C.; Fodor, S. P. A.; Adams, C. L.; Sundaram; Jacobs, J. W.; Schultz, P. G. *Science* **1993**, *261*, 1303.
6. Beck, F. *Methods in Molecular Biology*; Humana Press: Totowa, NJ, 2002; pp. 29–41.
7. Bunin, B. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1992**, *114*, 10997.
8. The roots of Ellman's work can be traced back to the pioneering work of Castells and co-workers, who described the first solid-phase synthesis of a benzodi-

- azepinone: Camps, F.; Castells, J.; Pi, J. *Anal. Quim.*, **1974**, 70, 848.
9. Nicolaou, K. C.; Pfefferkorn, J. A. *Biopolymers* **2001**, 60, 171.
 10. Sadler, K.; Tam, J. P. *Rev. Mol. Biotech.* **2002**, 90, 195.
 11. Merrifield, R. B. *Adv. Enzymol.* **1969**, 32, 221.
 12. Van den Nest, W.; Albericio, F. In *Optimization of Solid-Phase Combinatorial Synthesis*; Yan, B.; Czarnik, A. W., Eds.; Marcel Dekker: New York, 2001; pp. 91–107.
 13. Some authors tend to confuse the terms ‘*handle/linker*’ and ‘*solid support*’. The term *handle/linker* should be used to indicate the way in which the solid support has been functionalized. In some cases, such in the hydroxymethyl/Merrifield resin, the linker is already incorporated into the resin (the hydroxymethyl groups). In other cases, the *handle/linker* is a bifunctional spacer molecule, which becomes attached *permanently* to a functionalized resin at one end, often through a stable amide bond, and is linked *temporarily* to a growing molecule at the other end. The term solid support should be reserved exclusively to describe the polymer used. Thus, the solid support in the chloromethyl/Merrifield resin is polystyrene.
 14. Czarnik, A. W. *Biotechnol. Bioeng.* **1998**, 61, 77.
 15. Classical convergent strategies for dendrimer preparation start at the surface and go to the nucleus (from *out to in*).
 16. Crespo, L.; Sanclimens, G.; Royo, M.; Giralt, E.; Albericio, F. *Eur. J. Org. Chem.* **2002**, 1756.
 17. Crespo, L.; Sanclimens, G.; Montaner, B.; Pérez-Tomás, R.; Royo, M.; Pons, M.; Albericio, F.; Giralt, E. *J. Am. Chem. Soc.* **2002**, 124, 8876.
 18. Fromont, C.; Bradley, M. *Chem. Commun.* **2000**, 283.
 19. Lebreton, S.; Newcombe, N.; Bradley, M. EuroCombi-1. First Symposium of the European Society of Combinatorial Chemistry, Budapest (Hungary), July 1–5, 2001, poster.
 20. Lebreton, S.; Newcombe, N.; Bradley, M. *Tetrahedron Lett.* **2002**, 43, 2479.