Pergamon

Tetrahedron Letters 40 (1999) 407–410

TETRAHEDRON LETTERS

Preparation of Spin-Labeled Styrene-Divinylbenzene Copolymers and a New Approach for Quantitative Determination of the Resin Loading Using ESR Spectroscopy

Alan R. Katritzky,*[§] Sergei A. Belyakov,[§] Sonja Strah,[§] Brant Cage,[‡] and Naresh S. Dalal[‡]

[§]Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA

[‡]Department of Chemistry and National High Magnetic Field Lab, Florida State University, Tallahassee, FL 32306-3006, USA

Received 1 September 1998; accepted 27 October 1998

Abstract: Several spin-labeled carboxyl-containing resins were prepared, containing different concentrations of spin labels (nitroxyl or verdazyl stable free radicals). ESR studies of the prepared resin specimens demonstrated that the dependence between their ESR signal area and estimated and/or calculated amount of attached spin labels is linear in the case of verdazyl radicals, which makes this non-destructive approach a promising fast and accurate method for a resin loading determination.

Current analytical techniques used for solid-phase syntheses form a rather short list, the most important being ^{1}H and ^{13}C NMR, GC/MS and HPLC/MS.[1] At the beginning of combinatorial chemistry, FTIR was used for qualitative analyses of polymer-bound intermediates, but reliability has been a problem. NMR techniques (MAS, gel-phase) are presently the most frequently used analytical methods in combinatorial chemistry: the introduction of MAS ^{1}H NMR method is particularly promising, but NMR always needs support by an additional analytical method (MS and/or HPLC). In addition, the sensitivity limit of NMR spectroscopy (10^{-5}-10^{-6} mol) is not yet high enough for the routine analysis of organic libraries. Mass spectroscopy, although it can often be very sensitive (picomol scale), cannot yet compete (even including state-of-the-art modifications, such as ES-MS, MALDI, *etc.*)[2] with NMR as regards to structure elucidation, and always requires the cleavage of the products from the polymer support. Isotope tagging[3] and radiofrequency tagging,[2,4] recently reported as promising "noninvasive" encoding techniques for combinatorial chemistry, require expensive reagents or equipment.

The use of stable free radicals as spin labels in biochemistry of *natural* polymers (peptides or oligonucleotides) is well-known and has mainly concentrated on the study of chain mobility or interaction with surroundings (for instance, recent work deals with the use of spin labels and of the ESR method for monitoring the aggregation of peptide chain attached to polymer support).[5] By contrast, their use in the preparation of spin-labeled synthetic polymers was for strategically different purpose, *i.e.*, to study the magnetic (especially possible long-range order) interaction in anisotropic solids.[6,7]

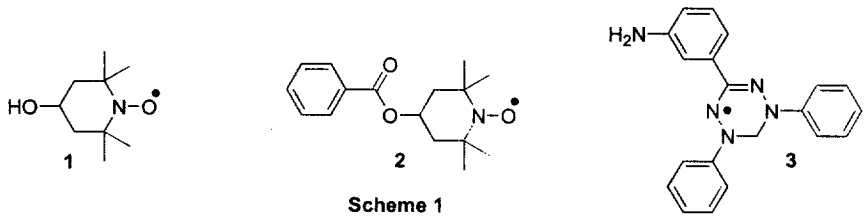
The successful use of spin labels in analysis of complex biological molecules and their synthetic analogs is based on high reliability, sensitivity, and reproducibility of ESR/EPR spectroscopy, which is a common tool for identification of paramagnetic labeled compounds. Solid and solution phase ESR spectra of stable free

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02382-X

radicals are both very characteristic and even allow analysis of radical mixtures. The sensitivity limit of ESR spectroscopy (10^{-9}-10^{-10} mol for the solid state and 10^{-13}-10^{-14} mol for solutions) is superior to many other analytical methods, especially to NMR. Finally, the ESR/EPR method is more rapid in micro scale analysis than NMR, which requires a long acquisition time: for instance, ^{13}C NMR resolution of polymer-bound sample usually is resolved only after several hours, while ESR of a spin-labeled polymer will take only a few minutes.

We have developed a new fast protocol for the determination of functionalized resin loading, based on the use of spin labels capable of binding to the solid supports and on the use of the ESR technique as a method of qualitative/quantitative control of such binding.

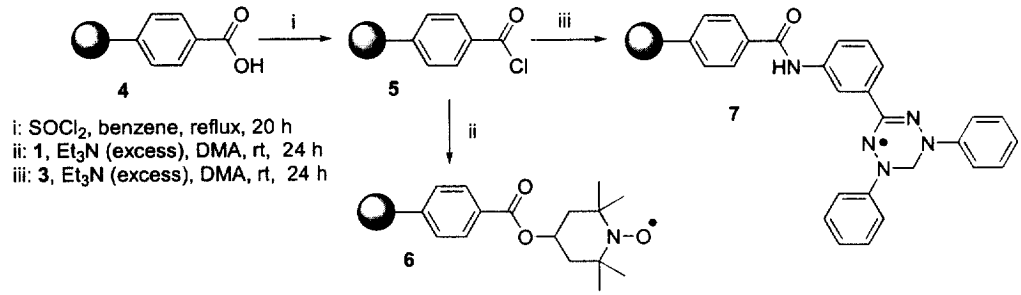
As an initial object, we chose the carboxy-containing copolymer of styrene/divinylbenzene (1%). We assumed that the preparation of a series of resins *via* attachment of various amounts of spin labels to the carboxy groups of the polymer matrix will mimic the situation when it is necessary to determine the loading of resins with different content of carboxy groups. 4-Hydroxy-TEMPO (**1**, Scheme 1) and 6-(3-aminophenyl)-2,4-diphenylverdazyl (**3**) were selected as spin labels, considering their distinct ESR spectra and the presence of reactive functional groups (hydroxy and amino, respectively). To prepare the calibration chart for the use of TEMPO spin label, we synthesized the model compound, 4-(benzoyloxy)-TEMPO (**2**), by reaction of **1** with benzoyl chloride in DMA in the presence of triethylamine.



Scheme 1

The calibration chart was obtained from the results of ESR measurements of a "blank"-labeled resin series, prepared by suspending of pre-weighed amounts of *unsubstituted* polystyrene-divinylbenzene (1%) co-polymer (0.1 g per sample) in dichloromethane aliquots containing different concentrations of **2** (0.5%, 1%, 5%, 10%, and 20% mass). After stirring overnight at room temperature, the solvent was evaporated in vacuum, the residues were dried in vacuum overnight, and the resulting *mechanical* mixtures of **2** and of unsubstituted co-polymer were analyzed by ESR spectroscopy (25 mg of solid sample of "blank"-labeled resin in each case). As expected, the integration of ESR signal area (which is proportionate to the concentration of radical spin centers) in the "blank"-labeled resins correlated linearly with the mass concentration of the model spin label **2** in such *mechanical* mixtures. We then prepared a series of resins containing *chemically* bound TEMPO radical. Carboxy-containing copolymer (Novabiochem, HL, 2.47 mmol/g) was converted into the corresponding acid chloride **5** (Scheme 2), according to the method of Leznoff *et al.*[8] The resin **5** was then treated with DMA aliquots containing different concentrations of nitroxyl radical **1** (1%, 5%, 10%, 20%, and 30% molar). Series of the resins **6** thus prepared which contained various amounts of TEMPO spin labels[9] were studied by the ESR spectroscopy. It was found that the integration of the ESR signal area increases linearly with the increase of nitroxyl radicals content *only* when the molar concentration of nitroxyl radicals is lower than 10%. At the higher concentrations of TEMPO label, the ESR signal of TEMPO-*bound* samples of polymers is broadened and its intensity is decreased. This phenomenon may be explained by the increasing spin-spin exchange of the neighboring TEMPO spin labels. Indeed, as Cilli *et al.*[5] also noticed recently in the

case of nitroxide labels, it was necessary to keep the radical concentration as low as possible in the labeling of peptide-containing resins to avoid spin-spin exchange which may interfere with the ESR line shapes.



Scheme 2

We then switched to verdazyl spin labels **3**,[10] which are noticeably more stable[11] but not as widely used as nitroxyls in labeling. Similarly to the TEMPO series, *mechanical* mixtures of 3-(3-aminophenyl)-1,5-diphenylverdazyl (**3**) as a model radical and blank styrene-divinylbenzene co-polymer were prepared, containing 1%, 3%, 5%, 10%, and 20% mass of spin labels on blank resin, and were analyzed by ESR spectroscopy in order to obtain the calibration chart which again linearly correlated the intensity/area of the ESR signal and the concentration of radical (Figure; last point, 20%, is omitted because of the space reason). Next, we prepared three resins **7** containing *chemically* bound verdazyl radicals by the reaction of resin **5** with **3** in different concentrations,[9] initially 4.1%, 8.1%, and 16.2% mass. The samples of spin-labeled resin **7** thus obtained (Scheme 2) were subjected to the ESR study.

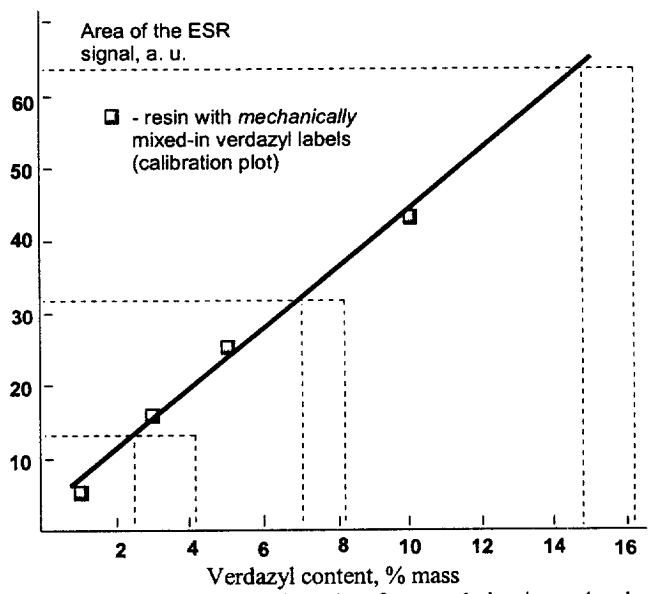


Figure. Calibration plot and results of extrapolation in verdazyl-labeled polymers

Results of the study are shown in the Figure. The determined and initially taken concentrations of radicals (left and right dotted lines in each case, respectively) have a discrepancy of about 1-1.5%, which reflects the incomplete conversion of the carboxy groups of resin **4** into the acid chloride groups of resin **5**. This leads to a lesser amount of verdazyl labels to be attached to the resins; however, the conversion is still very high. It is also clear that the dependence between the signal intensity/area and the amount of the attached verdazyl radicals is linear, which suggest that in the case of verdazyl spin label there is no such strong intra- or interchain spin-spin exchange as it was observed using TEMPO radical. Generally, the obtained data show that this approach has a potential for resolving of at least two important problems of solid phase chemistry:

(i) When a new polymer was prepared, which contains functional groups capable of binding the amino groups of verdazyl spin label **3** (*i.e.*, -COOH, -NCO, epoxy, *etc.*); this approach gives rapid and accurate way to determine the *actual* loading of such resins for the following of chemical reactions with aromatic amines.

(ii) In combinatorial chemistry, this approach holds great potential for qualitative control of sequential chemical transformations involving creation of the functional groups of the above type. Labeling of the newly created functionalized resin with a small amount of such spin label will provide a reliable tag which is ESR-detectable throughout all the combinatorial protocol. It is much less expensive than the radio-frequency method,[2,4] and can potentially be used for a single bead tagging.

The range of verdazyl spin labels can be easily expanded, depending on the types of functionalized resins needed to be analyzed. Our studies here are under way and will be reported in due course.

Acknowledgment. Financial support from the Slovenian Science Foundation in the form of a postdoctoral fellowship for S. S. is gratefully acknowledged.

REFERENCES AND NOTES

1. Fitch, W. L. Analytical Methods for the Quality Control of Combinatorial Libraries. In *Annual Reports in Combinatorial Chemistry and Molecular Diversity*, Volume 1; Moos, W. H.; Pavia, M. R.; Kay, B. K.; Ellington, A. D., Eds.; ESCOM Science Publishers: Leiden, 1997, 59-68.
2. Xiao, X.-y.; Nova, M. P. In *Combinatorial Chemistry: Synthesis and Application*; Wilson, S. R.; Czarnik, A. W., Eds.; John Wiley & Sons, Inc.: New York, 1997, 135-152.
3. Geysen, H. M.; Wagner, C. D.; Bodnar, W. M.; Markworth, C. J.; Parke, G. J.; Schoenen, F. J.; Wagner, D. S.; Kinder, D. S. *Chem. Biol.* **1996**, *3*, 679-688.
4. Nicolaou, K. C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2289-2291.
5. Cilli, E. M.; Marchetto, R.; Schreier, S.; Nakaie, C. R. *Tetrahedron Lett.* **1997**, *38*, 517-520.
6. Winter, H.; Gotschy, B.; Dormann, E.; Naarmann, H. *Synth. Met.* **1990**, *38*, 341-352.
7. Hmyene, M.; Naarmann, H.; Winter, H.; Pilawa, B.; Dormann, E. *J. Phys.: Condens. Matter* **1994**, *6*, L511-L515.
8. Leznoff, C. C.; Dixit, D. M. *Can. J. Chem.* **1977**, *55*, 3351-3355.
9. *A General Method for the Preparation of Resins **6** and **7**:* Solutions of **1** and **3** in DMA (50 mg/100 mL) were prepared. To the resin **5** (50 mg, estimated loading 2.47 mmol/g) in DMA (5-20 mL) the corresponding aliquotes of **1** or **3** (5, 10, 20% mol) and Et_3N (excess) were added. Mixtures were stirred at rt for 24 hours, resins were filtered, washed with THF-H_2O (1:1) (5 x 30 mL), THF (5 x 30 mL), CH_2Cl_2 (5 x 30 mL) and dried in vacuum overnight to give resins **6** or **7**.
10. Neugebauer, F. A.; Bernhardt, R. *Chem. Ber.* **1974**, *107*, 529-536.
11. Neugebauer, F. A. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 455-464.