Pergamon

Tetrahedron Letters 39 (1998) 753–756

TETRAHEDRON LETTERS

Monitoring Solid Phase Reactions with Ion-Selective Electrode

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Received 29 August 1997; revised 25 September 1997; accepted 7 November 1997

Abstract: A noninvasive method for monitoring and quantitative determination of basic functionalities ($pK_{HB}+ >7$) on solid support is described and its usefulness is demonstrated with practical examples. The method is based on quantitative protonation of the basic moieties on solid support with perchloric acid, followed by release of perchlorate anions by base and their concomitant potentiometric determination using an ion-selective electrode.

The intensity of effort that has been devoted over the last several years to the development of solid phase organic reactions reflects a great deal of interest in generating large collections of organic molecules for evaluation in lead discovery process. Simple and highly selective and sensitive methods suitable for fast and reliable monitoring of organic reactions on solid phase are an important aspect of efficient optimization and refinement of a variety of sequential transformations. Over the years, many techniques have been developed to provide both structural and quantitative information about solid phase-bound compounds. Spectroscopic methods which have proven very useful for monitoring solid phase organic reactions include techniques such as magic-angle spinning (MAS) for line narrowing in ^{1}H, ^{13}C and CH correlation NMR spectroscopy,[1] on-bead FTIR,[2] and MALDI/MS spectroscopy.[3] Since these techniques require quite expensive and specialized equipment, in most cases one is forced to rely on indirect methods, such as color tests,[4] spectrophotometric methods,[5] elemental analysis, functional group titration[6] or cleavage of a small sample from the support, in order to assess the fate of a particular reaction.[7] Other methods, particularly nonaqueous potentiometric titrations of basic functionalities with perchloric acid, have been successfully adapted to solid phase as well and incorporated into automatic peptide synthesizers.[8]

We report here a simple technique, routinely used in our laboratories, which allows real-time monitoring of solid phase reactions. This method is based on complete protonation of basic functionalities after treatment of the resin with a large excess of 1% $HClO_4$ which provides an easily detectable perchlorate anion. After thorough washing, the bound anion is eluted with a suitable base and quantified potentiomentrically with perchlorate ion-selective electrode (ISE).[9] The amount of basic component is calculated[10] from output potential that is logaritmically proportional to the activity of the selected ion in the sample phase via the Nernst equation: $\mathbf{E = E^0 + RT/z_iF\ ln(a_i)}$, where **E** is the cell potential measured in volts; $\mathbf{E^0}$ is the cell constant including the constant EMF contributions from inner and outer reference electrodes[11] as well as the inner-phase boundary potential of the membrane; $\mathbf{z_i}$ is the charge of the analyte ion; $\mathbf{a_i}$ is the analyte ion activity in the sample; **T** is the temperature in K; and **R** and **F** are the gas and Faraday constants, respectively. Since the activity of an ion is strongly influenced by the total ionic strength of the solution, a high and constant ionic strength relative to the measured ion concentration is required to ensure that the activity coefficient remains constant. Hence, the activity $\mathbf{a_i}$ becomes directly proportional to the concentration. This is achieved by adding ionic strength adjustor (ISA), a solution of $(NH_4)_2SO_4$, to all samples and standards.[12] With a constant ionic strength, the Nernst equation then reduces to: $\mathbf{E = E^0 + S*log(c)}$, where **S** = -2.303RT/F. There are several practical consequences of the simplified Nernst Equation: (1) For either one half or twice the concentration of any monovalent anion, there is a maximum difference in mV reading equal to |S*log2|. (2) Using the mV-meter with resolution of 0.1 mV, one should be able to read changes in concentration of 0.4%. However,

0040-4039/98/$19.00
PII: S0040-4039(97)10617-7

for practical purposes, a resolution of 1 mV will suffice. (3) Due to the dependence of electrochemical potential on temperature, samples and standards must be at the same temperature at the instant of the measurement. A 1°C difference in temperature will result in 3% error at the 10^{-4} M level.

Our first task was to establish the ISE approach as a viable and useful extension to existing monitoring methods. The general use of ISE was evaluated on reaction sequences leading to peptides, peptoids, and (poly)amines. In an initial model experiment, Fmoc-Gly-OH was coupled to a TentaGel S NH_2 resin followed by Fmoc deprotection and a subsequent coupling of bromoacetic acid. Nucleophilic displacement of bromine with benzylamine afforded H-(Bn)Gly-Gly-TG dipeptide which was further reacted with another Fmoc-Gly-OH and gave, after deprotection, the tripeptide H-Gly-(Bn)Gly-Gly-TG. As shown in Table 1, initial loading of the resin was in good agreement with the substitution declared by manufacturer as judged from Fmoc- (UV-active piperidine-dibenzofulvene adduct)[7] and ISE-readings. Completion of the peptide-peptoid formation was monitored by ISE and verified by the final Fmoc-reading after the quantitative[13] coupling of Fmoc-Gly-OH. Similarly, intermediates in a synthetic sequence leading to H-βAla-Phe(NO_2)-TG S RAM dipeptide were successfully monitored by ISE as shown in Table 1. Notably, the ISE method proved to be a convenient technique for determination of initial substitution for TentaGel-type resins. Furthermore, we tested the stability of carbonate and amino trityl linkers to prolonged exposures with $NaHCO_3$ (ISA solution) and 1% $HClO_4$, respectively. In both cases we did not observe any release of resin-bound compound from the solid support, confirming the noninvasive nature of this method.

Table 1 ISE Monitoring of Peptide/Peptoid Synthesis

Structure	ISE (mmol/g)	Fmoc (mmol/g)
H_2N-TG	0.27±0.01[a]	NA
H-Gly-TG	0.26±0.02	0.26
$BrCH_2CO$-Gly-TG	0.00	NA
H-(Bn)Gly-Gly-TG	0.25±0.02	NA
H-Gly-(Bn)Gly-Gly-TG	0.25±0.02	0.25
H_2N-TG S RAM	0.19±0.01[b]	NA
H-Phe(NO_2)-TG S RAM	0.18±0.02	0.19
H-βAla-Phe(NO_2)-TG S RAM	0.19±0.02	0.19

[a] Declared S = 0.29 mmol/g; [b] Declared S = 0.20 mmol/g.

Another practical example of ISE monitoring is related to our ongoing project in solid-phase synthesis of secondary and tertiary amines.[14] A reliable method for monitoring the progress of amine formation was of particular importance for this project, since the essential feature of this system, as indicated in Table 2, is the change in the number of amine functionalities during the synthesis. In order to monitor relative changes in the basic functionality content, we introduced an internal standard (isolated tertiary amine) between the solid support and HMPB linker. Attachment of N,N'-dimethylethylenediamine to the HMPB linker **4** via carbamate linkage afforded resin-bound amine **5**. From ISE-reading, it was clear that all reaction steps proceeded with nearly quantitative conversion. Palladium(0)-mediated N-alkylation in the following step could not be monitored due to the unchanged number of amino groups. However, esterification of aminoalcohol **6** with Fmoc-Gly-OH in the next step indicated nearly quantitative N-alkylation of **5** as well as esterification of **6**. Additional supporting data for transformation of **5** → **6** came from the Fmoc-reading after treatment of the Fmoc protected amine with 50% piperidine/DMF. Interestingly, ISE-monitoring of the final cleavage step revealed incomplete detachment of desired triamine from the HMPB linker using mixture of 5% iPr_3SiH in 95% TFA/water for 2 h. Assuming stability of ester and amide bonds toward acidic treatment, the estimated overall yield of correct product **8** (**4** → **8**) was about 44 %.[15]

In the course of our investigations we have also noticed some limitations in quantitative determination of basic functionalities. One obvious problem inherent in this method is a salt hydrolysis effect. Since the procedure for perchlorate determination involves repetitive washings with distilled water, solvolysis of ammonium salt will occur, which is dependent upon the dissociation constant of the protonated base and the autoprotolytic constant (pK_{AP}) of the solvent used. In general, as can be expected from the corresponding pK_{HB+}'s , most primary, secondary, and tertiary amines, amidines, guanidines, imidazoles, and electron rich

Table 2 ISE Monitoring of Polyamine Synthesis with an Internal Amine Standard

No.	Structure	ISE Reading (mmol/g)
1	H_2N-TG	0.19±0.01[h]
2	a → Br, NH-TG, O	NA
3	b → HN, $(CH_2)_4$, N, NH-TG, O	0.36±0.03
4	c → CH_3O, HO, O, $(CH_2)_3$, N, $(CH_2)_4$, N, NH-TG, O; Linker	0.18±0.02
5	d → HN, N, O–Linker-NHTG, O	0.39±0.02
6	e → HO, N, N, O–Linker-NHTG, O	0.36±0.02
7	f → H_2N, O, N, N, O–Linker-NHTG, O	0.50±0.03[i]
8	g → H_2N, O, N, NH + 4	0.34±0.02[j]

[a] $BrCH_2COOH$, DCC, 2 x 2 h; [b] $MeHN(CH_2)_6NHMe$, DMSO, 16 h; [c] HMPB, DIC, HOBt, 16h; [d] 1. CDI, THF, 2h, 2. $MeHN(CH_2)_2NHMe$, NMP, 50°C, 16 h; [e] 4-vinyl-1,3-dioxolan-2-one, $Pd(PPh_3)_4$, THF, 5 h; [f] 1. FmocGly-OH, DIC, HOBt, NMI, DMF, 16 h, 2. 50% piperidine in DMF, 20 min; [g] 5% of iPr_3SiH in 95% TFA/water, 2 h; [h] An average of four independent measurements; [i] Fmoc reading from Fmoc-7 was 0.16 mmol/g; [j] After 7 days treatment with 95% TFA/water ISE-reading was unchanged.

dialkylaminobenzenes behave normally in aqueous solution whereas pyridines and anilines are not suitable basic functionalities for quantitative determination. Notably, indol-type compounds are completely "invisible" with such a technique in an aqueous solution. In order to cover other basic functions such as substituted anilines and pyridines, solvents which are less basic than water must be used. Unfortunately, we have not been able to obtain consistent data for TentaGel-type resins with solvents such as acetic acid, ethylene glycol/iPrOH, acetonitrile, and methyl isobutyl ketone. This is presumably due to the proton complexation by polyethyleneglycol units within the polymer beads. Electrode readings were in the range of 300-700% of theoretical resin substitution, suggesting "trapping" of perchlorate anions by the polymer.

Also, attempts to extend the method to aminomethylphenyl and p-methylbenzhydrylamine (MBHA) type of resins were not successful. Unfavorable swelling properties of these resins in aqueous media and a low tolerance of the ISE to organic solvents required modified measurement methods which produced unreliable results. In this experiment, water-miscible solvents (iPrOH, MeOH, THF, dioxane, CH_3CN, DMA) were evaluated for both resin types to confirm the declared substitution level. Fmoc-Gly-OH was then coupled to the same samples and the values obtained by ISE- and Fmoc-readings were compared for each resin sample. Final washings of perchlorate anions were performed with 2% ammonia in the test solvent and a final concentration about 8-10% of organic solvent in ISA. A time dependence in the ISE-reading was observed for all samples, indicating an adverse effect of the organic solvents on the sensing electrode membrane.

In conclusion, ISE methodology can be used to quantify a wide variety of basic functionalities and their relative changes during a reaction on solid support. Limitations of this methodology include the requisite use of water-compatible solid supports and basic functionalities possessing a pK_{HB+} within a certain range. A significant advantage of the described method is that it is simple and noninvasive when compared to traditional "cleave and characterize" techniques. The concept of using ISE to monitor progress of reactions on solid phase certainly offers another attractive tool to solid phase organic and peptide chemists.

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9. (a) Morf, W.E. *The Principles of Ion-Selective Electrodes and of Membrane Transport*; Elsevier: Amsterdam, 1981. (b) Solid phase syntheses of single compounds as well as monitoring steps involving treatment with 1% $HClO_4$ followed by multiple washings were performed in polypropylene syringes equiped with polyethylene frits.
10. For easier calculation of concentration and error estimates, Microsoft Excel templates can be downloaded from the Molecular Diversity Webpage at http://www.5z.com/.
11. Use of a combination electrode does not require an additional reference electrode since the indicating half-cell and reference half-cell are joined coaxially in a single body.
12. 0.33M aqueous solution of $(NH_4)_2SO_4$, which corresponds to ionic strength I=1M, is recommended for 1-50 mg of resin with loading 0.2-0.3mmol/g.
13. Chloranil test was used to comfirm completion of the last coupling step (see, ref. 7).
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15. Purity of the product **8** after final cleavage was > 95% (HPLC, ^1H NMR). Structure identity was further confirmed by MS.