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Use of an Internal Reference for the Quantitative HPLC-UV Analysis of Solid-Phase Reactions: A Case Study of 2-Chlorotrityl Chloride Resin

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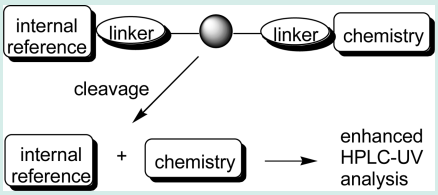
Supporting Information

ABSTRACT: Here we evaluated the use of internal reference compounds for the rapid assessment of reactions performed in solid-phase. An internal reference compound (commercially available) was bound to the resin, together with the substrate, and cleaved with the products after completion of the reaction. The peak area of the reference compound in the HPLC-UV chromatograms can be correlated directly with those of other compounds present in the reaction mixture, thereby allowing a quantitative interpretation of the chromatograms with respect to conversion and yield. The usefulness of this method was demonstrated by optimization of a protocol for the synthesis of proline-based tripeptides.



KEYWORDS: *internal standard, reporter compound, analytical construct, Barlos-Resin, DKP-formation*

Solid-phase synthesis is widely used in several fields of chemistry, such as combinatorial sciences, organic, and peptide chemistry.[1,2] However, the optimization of reactions performed on solid-phase requires considerably more effort than when performed in solution. Essential information like the conversion of starting material and product yield cannot normally be obtained directly. NMR, IR, and mass spectroscopy methods applied to compounds on solid supports seldom provide information about quantitative composition.[3] The outcome of organic reactions on solid supports (usually resins of organic polymers) is commonly monitored by HPLC-UV analysis of the products obtained from a cleaved resin sample.

The content of a compound is usually expressed as a percentage of its peak area from the total peak area. However, UV-absorption coefficients can differ from compound to compound. In this case, the relations of the area values of the different peaks do not directly reflect the molar concentrations of the corresponding compounds.[4] This makes it difficult to construe chromatograms from reaction mixtures involving, for example, strongly absorbing protecting groups.

A second limitation of the conventional quantification is that the exact amount of resin the sample was prepared from is not known. Consequently, the peak areas in a chromatogram from a test reaction performed on a resin sample cannot be correlated with the peak areas in a chromatogram obtained from another reaction run on another sample of the same resin.

A theoretically simple solution to these problems might be the cleavage of the samples from resin aliquots and the addition of a fixed amount of (an external) reference compound. However, there is no straightforward way to obtain resin aliquots from reaction mixtures. In the course of a synthesis, the resins are swollen in the solvents and drying until weight constancy causes a significant delay for the evaluation of the outcome of a reaction. Furthermore, the molecular weight of the resin-bonded compounds changes during synthesis, and consequently, so does the resin weight. An alternative solution would be to run the reactions separately on a mini-scale on resin aliquots until an analysis is required. Consequently, all the resin from a reactor has to be cleaved. Prior samples (for intermediate analysis) cannot be taken. Generally, strategies that involve measuring the exact amount of resin subjected to cleavage require much more material than for HPLC-analysis (1−5 mg). Moreover, they are error-prone and imply time-consuming sample preparation for each analysis.

Resins equipped with analytical constructs provide great improvements (Figure 1A). Such a reporter resin can be mixed in a small amount with the resin on which the synthesis is performed. The analytical constructs have two orthogonal cleavage sites. By analytical cleavage, conversion of starting material, yield and identity can be unambiguously determined as a result of the presence of the analytical construct bound to the products, which combine a chromophore that absorbs at a remote wavelength and a mass-sensitizer. For the synthesis, the

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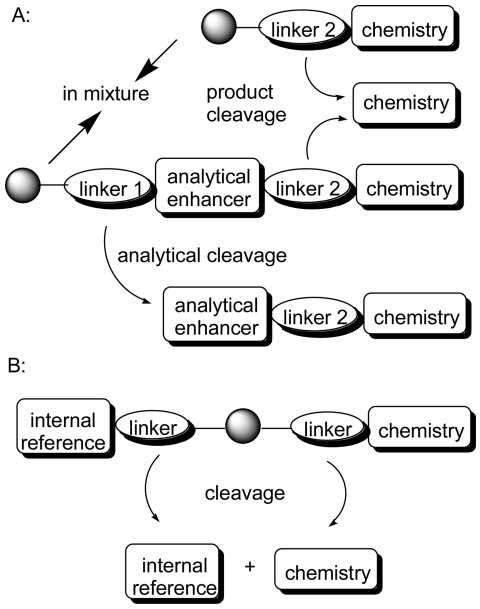

Figure 1. Analytical construct resin with two orthogonal cleavage sites is mixed with the resin used for the synthesis (A). An internal reference can be introduced as "willful contamination", together with the starting material, in the resin-loading step. This resin can be used to optimize the reaction conditions (B).

product can be cleaved without analytical construct. However, such useful tools are not readily available, and so far have been developed for the preparation of carboxamides only.[5,6] It should also be considered that this approach can lead to erroneous results because the outcome of reactions on the reporter-resin with its extra-linker should not be the same as on the resin used for the synthesis.

Here we report a strikingly simple and straightforward method for the quantitative analysis of reactions performed on solid phase (Figure 1B). A reference compound is bound to the resin, together with the starting material, via the same functional group and is cleaved under the same conditions as the products.[7] Commercially available compounds can be used as reference. A suitable reference is chemically inert to the reaction conditions applied. Its HPLC-retention time differs from those of the other compounds and the reference substance can be detected at the same wavelength used for the UV detection of products. This strategy is expected to be applicable to all types of resins and linkers, and the synthetic steps will proceed on the same linker and resin as used for the synthesis. The area of product peaks in HPLC chromatograms can be expressed as multiples of the reference peak area and allows for comparison of chromatograms. Here we describe the use of an internal reference for the straightforward optimization of a protocol for combinatorial synthesis.

FOLLOWING A SOLID-PHASE PEPTIDE SYNTHESIS WITH AN INTERNAL REFERENCE

As proof of principle, we synthesized the tripeptide Val-Arg-Phe in the presence of 1-pyreneacetic acid (PAA) as reference compound. PAA is commercially available and can be easily identified because of its characteristic PDA-absorption profile. Its peak retention time was separated from those of the products obtained during the synthesis. As a simple carboxylic acid, PAA was expected to bind to the resin as stably as amino acids and peptides and be cleaved under the same conditions.

As solid support, we used 2-chlorotrityl chloride (CTC) resin, a standard support for the synthesis of peptides, pseudopeptides and nonpeptide molecules.[8,9] It was expected that the synthesis of Val-Arg-Phe on this resin would work well under standard conditions.

The solid-phase peptide synthesis (SPPS) started with the loading of CTC resin with Fmoc-Phe containing 10 mol % PAA (Figure 2). A test cleavage from the resulting resin **1** gave

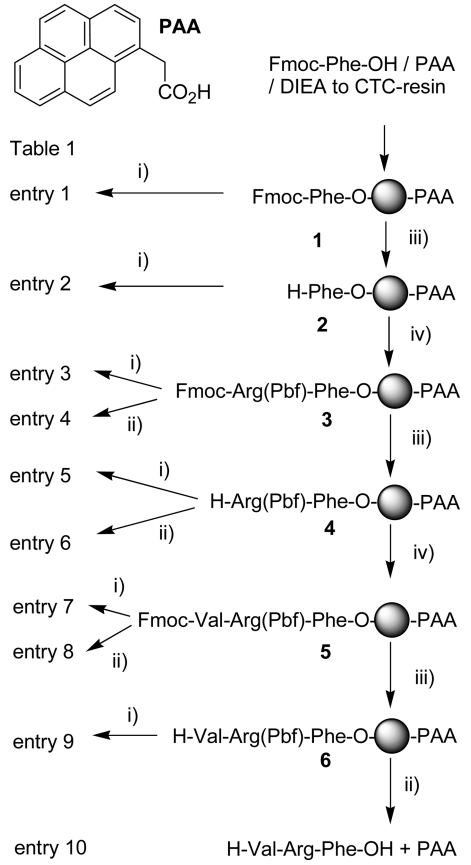

Figure 2. Standard SPPS of the tripeptide Val-Arg-Phe in the presence of PAA as internal reference. The results of intermediate product cleavages are summarized in Table 1. (i) 2% TFA in DCM, 10 min; (ii) TFA/H_2O (95:5), 90 min; (iii) 20% piperidine in DMF, 10 min.; (iv) 4 equiv Fmoc-Xaa, 4 equiv Oxyma, 4 equiv DIPCDI in DMF.

a HPLC-chromatogram with two peaks at 220 nm (a wavelength routinely used for product detection), namely the small PAA peak and the peak of Fmoc-Phe, the latter with a 15-fold area with respect to the former (Table 1, entry 1). We obtained the same result for a repeated test cleavage. The relation between the peak areas remained constant as long as the main peak was not higher than one absorption unit (1 AU). Therefore, we ensured that the main peak did not exceed 1 AU for all the HPLC chromatograms.

The Fmoc-group was removed from resin **1**, and a cleaved sample from the resulting resin **2** showed two peaks, corresponding to Phe and PAA, with nearly equal area values (Table 1, entry 2). The Fmoc-group exerts strong absorbance, and with regard to the reference signal, its impact can now be roughly estimated as a 15-fold increase in the peak area. Throughout this manuscript, we will express the term "*x*-fold multiple of the peak area respectively to the reference peak area" by "area ratio". Thus, an area ratio of 15 implies that the

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Table 1. Summary of Product Compositions from Intermediate Cleavages during the Synthesis of Val-Arg-Phe (Figure 3)

		area ratio[b]			
entry[a]	product	Ph, CONH	Ph, CONH, +Fmoc	Ph, CONH, +Pbf	Ph, CONH + Fmoc, +Pbf
1	Fmoc-Phe		15		
2	Phe	1.0			
3	Fmoc-Arg(Pbf)-Phe				46
4	Fmoc-Arg-Phe		14		
5	Arg(Pbf)-Phe			30	
6	Arg-Phe	1.8			
7	Fmoc-Val-Arg(Pbf)-Phe				43
8	Fmoc-Val-Arg-Phe		13		
9	Val-Arg(Pbf)-Phe			30	
10	Val-Arg-Phe	2.2			

[a]See Figure 3. [b]Area ratio means the multiples of the PAA (reference) peak area; for chromatograms see Supporting Information.

peak area of the analyte is 15 times higher than that of the reference compound.

After coupling Fmoc-Arg(Pbf) to **2**, the chromatogram of a sample from the resulting resin **3** shows that the Fmoc-Arg(Pbf)-Phe area ratio was nearly 46 (Table 1, entry 3). This increase resulted from absorption of the Fmoc- and the Pbf-group. The contribution of the former was expected to produce an approximately 15-fold increase in absorption, so we assumed that the additional 30-fold increase was caused by the Pbf-group. To test this notion, resin **3** was treated with 95% TFA. Under these conditions, the Pbf-group was removed to give Fmoc-Arg-Phe with an area ratio of 14 (Table 1, entry 4). The Fmoc-group from **3** was removed to give resin **4**, and the area ratio of Arg(Pbf)-Phe was found to be 30 (Table 1, entry 5). Summing the contributions of the absorbance of the Pbf- and Fmoc-group (30 and 14) gave 44. This value is in good agreement with the 46 found for the dipeptide Fmoc-Arg(Pbf)-Phe (entry 3).

Treatment of resin **4** with 95% TFA yielded the dipeptide Arg-Phe, whose area ratio was 1.8 (Table 1, entry 6). The increase with respect to Phe (entry 2) can be attributed to the additional peptide bond.

Coupling of Fmoc-Val to resin **4** rendered resin **5**. A comparison of the area ratios of Fmoc-Val-Arg(Pbf)-Phe, Fmoc-Val-Arg-Phe, Val-Arg(Pbf)-Phe, and Val-Arg-Phe (entries 7, 8, 9, and 10, respectively) with the those of the products from the previous coupling cycle (Fmoc-Arg(Pbf)-Phe, Fmoc-Arg-Phe, Arg(Pbf)-Phe, and Arg-Phe; entries 3, 4, 5, and 6, respectively) shows that these areas did not increase as expected, but remained constant or even decreased. This finding indicates the loss of some product. In other experiments (vide infra), we found evidence that the activation of amino acids with oxyma/DIPCDI, as used throughout this synthesis, is not optimal for CTC resin.

The synthesis of the tripeptide H-Val-Arg-Phe-OH in the presence of a reference compound (PAA) allowed us to obtain a series of HPLC-UV chromatograms, wherein the products peak areas were quantified as multiples of the peak area of the reference compound. This approach allows for a coherent interpretation of chromatograms during a synthetic sequence that involves strongly absorbing temporary protecting groups.

USE OF AN INTERNAL REFERENCE FOR OPTIMIZING A SOLID-PHASE PROTOCOL FOR COMBINATORIAL SYNTHESIS

Here we demonstrated the use of a reference compound for the optimization of the synthesis of a library of tripeptides type Yaa-Xaa-Pro. The target peptides have Pro at the C-terminus and a rather sterically crowded amino acid (Yaa) at the N-terminus. We aimed to design a straightforward general protocol that works reliably with L- and D-, as well with sterically hindered amino acids in the middle position (Xaa).

Figure 3 shows the envisaged synthesis of three model peptides Fmoc-Aib-Gly-Pro, Fmoc-Aib-D-Leu-Pro, and Fmoc-Aib-Sar-Pro by stepwise SPPS in the presence of a reference compound. Aib represents the sterically crowded amino acid Yaa. The foreseeable critical step in the synthesis is the coupling of Fmoc-Aib to the sequence Xaa-Pro. It is known that dipeptides with C-terminal Pro form diketopiperazines (DKP) and that SPPS of these peptides is often hampered by this unwanted side-reaction, which can cause dramatic reduction in yield.[10] The DKP-prone sequences Gly-Pro and D-Leu-Pro are representative examples for DKP-formation (cyclo-Gly-Pro and cyclo-D-Leu-Pro) under the basic conditions of Fmoc-removal. Furthermore, an appropriate protocol should also give satisfactory results for sterically hindered couplings, which are represented by the coupling of Fmoc-Aib to the sterically hindered *N*-methylamino group on Sar-Pro-resin (Figure 3).

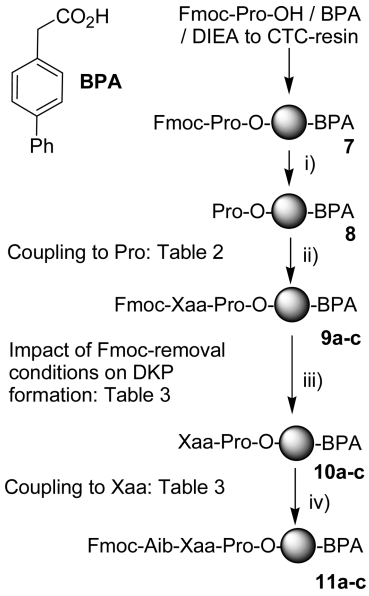

Figure 3. Stepwise SPPS strategy for the synthesis of compounds Fmoc-Aib-Gly-Pro, Fmoc-Aib-D-Leu-Pro, and Fmoc-Aib-Sar-Pro on CTC resin. Intermediate samples for HPLC-UV analysis were cleaved from resins **7**, **9a–c**, and **11a–c** with 2% TFA in DCM for 10 min ((**a**) Xaa = Gly, (**b**) Xaa = DLeu, (**c**) Xaa = Sar). The results (Tables 2 and 3) indicate that the following protocols show the best performance: (i) 2 × 10 min 20% piperidine in DMF, wash with DCM; (ii) 40 min 3 equiv Fmoc-Xaa activated with HBTU/DIEA, wash with DCM; (iii) 5 min 20% piperidine in DMF, wash with DCM; (iv) 40 min 3 equiv Fmoc-Yaa activated with HATU/DIEA, wash with DCM.

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We show that an internal reference contributed to obtaining a reliable estimation of the extent of DKP-formation and product yield under a range of coupling conditions, and thus indicates the best protocol for library synthesis.

CTC resin was used as solid support because it prevents DKP formation.[11] Biphenyl acetic acid (BPA) was found to have a peak separable from the product peaks (PAA coelutes with some products). One gram of CTC resin with a maximal capacity of 1.6 mmol/g was loaded with 0.3 mmol BPA and 0.8 mmol Fmoc-Pro. The area ratio between Fmoc-Pro and BPA and was 6.5 in a test cleavage from the test resin **7** (Table 2, entry 1).

Table 2. Coupling Conditions for the Synthesis of Resins 9a−c

entry	protocol applied to **8**	area ratio[a] (product)
1		6.5 (Fmoc-Pro from **7**)
2	Fmoc-Gly/Oxyma/DIPCDI, 40 min	3.7 (Fmoc-Gly-Pro)
3	Fmoc-Gly/HOAt/DIPCDI, 40 min	3.5 (Fmoc-Gly-Pro)
4	Fmoc-Gly/HBTU/DIEA, 40 min	6.8 (Fmoc-Gly-Pro)
5	Fmoc-Gly/HBTU/DIEA, 40 min	6.8 (Fmoc-Gly-Pro)
6	Fmoc-D-Leu/HBTU/DIEA, 40 min	7.2 (Fmoc-D-Leu-Pro)
7	Fmoc-Sar/HBTU/DIEA, 40 min	6.9 (Fmoc-Sar-Pro)

[a]Area ratios corresponding to **BPA**.

After removal of the Fmoc-group, a Pro-peak from resin **8** was not observed by HPLC-UV analysis and consequently its conversion to Fmoc-Xaa-Pro could not be followed directly. However, it is reasonable to expect that the area ratio of the subsequent product Fmoc-Gly-Pro from resin **9a** would be similar to that of Fmoc-Pro from **7**. Thus, in this case, the area ratio should be 6.5 or higher due to the additional amide bond. Coupling of Fmoc-Gly activated with oxyma/DIPCDI to resin **8** gave an area ratio of 3.7 (Table 2, entry 2), which did not improve by a repeated coupling. A similar result was obtained for HOAt/DIPCDI activation (area ratio 3.5, Table 2, entry 3). In contrast, by HBTU/DIEA activation, the product was detected with an area ratio of 6.8 (Table 2, entry 4). Since the yield did not increase by longer coupling time or by repeated coupling, we reasoned that the coupling under this condition proceeded quantitatively and that the Oxyma/DIPCDI and HOAt/DIPCDI activation conditions did not work well for coupling to **8**. The first part of the synthesis was then accomplished by coupling Fmoc-Gly, Fmoc-DLeu and Fmoc-Sar activated by HBTA/DIEA to give resins **9a−c**, from which Fmoc-Gly-Pro, Fmoc-D-Leu-Pro, and Fmoc-Sar-Pro were obtained with area ratios higher than the 6.5 (Table 2, entries 5, 6, and 7).

The next step was the removal of the Fmoc-group from the resins **9a−c**. DKP formation can proceed rapidly under these basic conditions (20% piperidine in DMF). It was not possible to obtain area ratios for the products Xaa-Pro from resins **10a−c**, because of the peak shape of the corresponding dipeptides. Fmoc-removal was subsequently followed by coupling of Fmoc-Aib activated by HBTU/DIEA for 40 min over resins **10a−c**. The products Fmoc-Aib-Gly-Pro (from **11a**) and Fmoc-Aib-D-Leu-Pro (from **11b**) were obtained with area ratios lower than the corresponding starting materials and these ratios did not increase with coupling time (Table 3, entries 1 and 2 vs Table 2, entries 5 and 6, respectively). This finding indicates a yield decrease during Fmoc-removal. In contrast, the coupling of Fmoc-Aib over resin **10c** only reached an area ratio of 2.0 after 40 min, but increased after 2 h of reaction time to 3.6 (Table 3, entries 3 and 4). This result indicates a slow coupling between the *N*-methyl group of Sar and Fmoc-Aib.

Table 3. Coupling of Fmoc-Aib over Resins 9a−c

entry	conditions[a,b]	area ratio[c] (product from resin)
1	[a]Fmoc-Aib/HBTU/DIEA, 40 min	6.4 (Fmoc-Aib-Gly-Pro, **11a**)
2	[a]Fmoc-Aib/HBTU/DIEA, 40 min	5.9 (Fmoc-Aib-D-Leu-Pro, **11b**)
3	[a]Fmoc-Aib/HBTU/DIEA, 40 min	2.0 (Fmoc-Aib-Sar-Pro, **11c**)
4	[a]Fmoc-Aib/HBTU/DIEA, 2 h	3.6 (Fmoc-Aib-Sar-Pro, **11c**)
5	[a]Fmoc-Aib/HATU/DIEA, 40 min	6.2 (Fmoc-Aib-Gly-Pro, **11a**)
6	[a]Fmoc-Aib/HATU/DIEA, 40 min	6.0 (Fmoc-Aib-D-Leu-Pro, **11b**)
7	[a]Fmoc-Aib/HATU/DIEA, 40 min	7.4 (Fmoc-Aib-Sar-Pro, **11c**)
8	[b]Fmoc-Aib/HATU/DIEA, 40 min	5.3 (Fmoc-Aib-Gly-Pro, **11a**)
9	[b]Fmoc-Aib/HATU/DIEA, 40 min	3.1 (Fmoc-Aib-D-Leu-Pro, **11b**)
10	[b]Fmoc-Aib/HATU/DIEA, 40 min	7.4 (Fmoc-Aib-Sar-Pro, **11c**)

[a]Prior Fmoc-removal by 20% piperidine in DMF for 5 min. [b]Prior Fmoc-removal by 20% piperidine in DMF for 20 min. [c]Area ratios corresponding to **BPA**.

The activation reagent HBTU was replaced by its more potent aza-analog HATU. The couplings over resins **10a** and **10b** rendered the similar area ratios as when HBTU was used (Table 3, entries 1 vs 5, and entries 2 vs 6, respectively). The coupling of Fmoc-Aib over **10c** gave an area ratio of 7.4 for Fmoc-Aib-Sar-Pro (Table 3, entry 7), which was higher than those obtained for the Gly (6.2) and D-Leu-analogs (6.0) (Table 3, entries 5 and 6). The area ratio did not increase with prolonged coupling. We therefore reasoned that after 40 min the coupling was complete and that the differences between the product area ratios corresponded to the susceptibility of the corresponding Xaa-Pro-sequence on resins **10a−c** to DKP formation under the Fmoc-removal conditions.

To test this, resins **9a−c** were treated for 20 min with 20% piperidine in DMF, and Fmoc-Aib was coupled for 40 min with HATU/DIEA activation. The highest impact of the prolonged basic treatment was observed for the DLeu-Pro sequence, which experienced a 50% reduction in yield (Table 3, entry 6 vs entry 9). The sequence Gly-Pro was also obtained in significantly lower yield (Table 3, entry 5 vs entry 8). However, the same yield was achieved for the sequence Sar-Pro (Table 3, entry 7 vs entry 10).

STABILITY OF CARBOXYLIC ACIDS BOUND TO CTC RESIN UNDER A RANGE OF ACYLATION CONDITIONS

Low yields of Fmoc-Gly-Pro were obtained when coupling Fmoc-Gly activated with Oxyma/DIPCDI over resin **8** (Table 2, entry 2). These conditions also caused a yield decrease in the synthesis of Val-Arg-Phe (Table 1). To check the stability of the reference compounds PAA and BPA, and peptides on CTC resin toward different acylation conditions, CTC resin was loaded with an equimolar mixture of BPA, PAA, Fmoc-Gly, Fmoc-Pro, and Fmoc-Leu (0.1 mmol each for 500 mg CTC resin) to give resin **12** (Figure 4).

Aliquots of washed and dried **12** (10 mg) were used to monitor the stability of the resin-bound compounds toward a range of activation conditions for Fmoc-Gly coupling to give **13**. After reaction and cleavage of the corresponding products from the resin aliquot, a fixed volume of an external reference (Z-Gly) solution was added. The area ratios of the products obtained from **13** are shown in Table 4 and refer to the peak area of the external reference (Z-Gly). The area ratios

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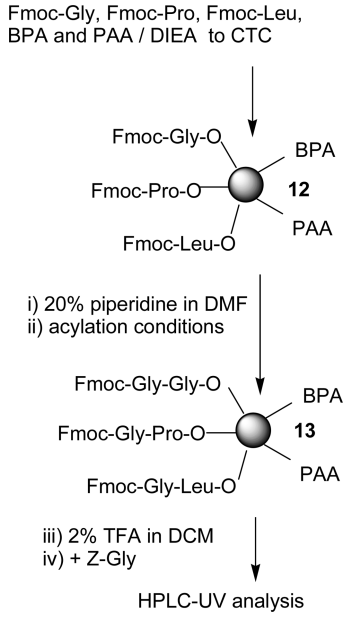

Figure 4. For conditions (ii) see Table 4.

corresponding to the Fmoc-Gly-Xaa-peptides and internal references (BPA, PAA) after coupling of Fmoc-Gly activated with HBTU/DIEA for 40 min were set to 100% (Table 4, entry 1). For all other conditions, the compound peak areas were smaller with respect to the conditions of entry 1 (Table 4, entries 2−8, the losses of compounds from resin with respect to entry 1 are given in parentheses). The yields dropped in all examples during "overnight"-couplings. The reduction in yield was dramatic (up to 60%) when acidic coupling reagents (Oxyma, HOBt and HOAt) were used (Table 4, entries 3−8). The reference compound PAA was found to be the most stable but in contrast, Fmoc-Gly-Pro on CTC showed the lowest stability under the conditions tested. Because of the similar and even higher stability of the reference compounds PAA and BPA with respective to peptides, we were able to detect the decreases in yield in the test reactions described above (Figures 2 and 3). With respect to SPPS on CTC resin, on the basis of our findings we recommend that DIPCDI-mediated HOAt, HOBt, and Oxyma-activation be carefully checked.[12]

SUMMARY

The data presented in this study demonstrate the internal reference method to be a valuable tool for optimizing conditions for solid-phase peptide synthesis and its possible applications in solid-phase synthesis of other compounds. We wish to summarize how to use this method.

First, it must be noted that the internal reference does not improve the chromatographic properties of resin-bound compounds. Its exactness depends on the integration of the peaks. With a suitable internal reference, reaction control by quantitative HPLC-UV analysis proceeds in the same manner as conventional HPLC-UV analysis (taking a resin sample from the reaction mixture, washing it, and running an HPLC chromatogram directly from the cleaved products). The practical value of an internal reference is the rapid sample analysis, which allows comparison of chromatograms from resin samples before and after reaction, or from different reaction conditions. Rather than providing "absolute" values for single chromatograms, the validity of this approach is based on the comparison of chromatograms.

When improvement of a solid-phase synthesis is required, a test resin with internal reference can be designed. There is no "general" internal reference available. However, here PAA and BPA, with high retention times in RP-HPLC, were found to be useful in peptide synthesis on CTC resin. As demonstrated, these compounds bound to the resin more stably or as stable as the peptides by the same functional group. They gave sharp peaks on HPLC-UV which were well separated from product peaks. However, there are no limitations for other compounds that fulfill these key requirements.

For quantification, the main peak should not exceed 1 AU to have a linear relation between the peak areas of the reference and the analyte. As a result, the area ratio between products and the reference should not be too large. The area ratios in this study were between 1 and 50. All analyses should be performed on the same equipment. The peaks have to be clearly distinguished from the baseline. Noisy baselines (caused by UV lamps with long running time) affect the accuracy.

We have demonstrated that such a quantitative analysis provides information rapidly and precisely on the outcome of reactions that would otherwise be difficult to obtain. Once the appropriate protocol is found, the synthesis can then be performed on a resin without a reference. Therefore we consider the internal reference method a valuable addition to the toolbox for solid-phase synthesis.

Table 4. Quantification of Premature Cleavage of Compounds on CTC Resin under a Range of Peptide Coupling Conditions (HBTU/DIEA, 40 min and 15 h, entries 1 and 2; oxyma/DIPCDI, 40 min and 15 h, entries 3 and 4; HOBt/DIPCDI, 40 min and 15 h, entries 5 and 6; HOAt/DIPCDI, 40 min and 15 h, entries 7 and 8)

	area ratio[a] (percent variation respectively to entry 1)				
entry	Fmoc-Gly-Gly	Fmoc-Gly-Pro	BPA	Fmoc-Gly-Leu	PAA
1	3.1 (±0%)	3.2 (±0%)	1.3 (±0%)	3.3 (±0%)	1.9 (±0%)
2	2.9 (−8%)	3.0 (−4%)	1.3 (−4%)	3.2 (−3%)	1.9 (+1%)
3	3.1 (−1%)	2.6 (−19%)	1.4 (+1%)	3.1 (−4%)	1.9 (±0%)
4	2.7 (−13%)	2.3 (−26%)	1.3 (−7%)	2.8 (−14%)	1.9 (±0%)
5	3.0 (−4%)	1.9 (−41%)	1.4 (+1%)	3.0 (−7%)	2.0 (+7%)
6	2.5 (−21%)	1.7 (−47%)	1.2 (−9%)	2.6 (−20%)	1.8 (−3%)
7	2.9 (−8%)	1.9 (−39%)	1.3 (−1%)	2.7 (−18%)	1.9 (+4%)
8	1.8 (−43%)	1.2 (−63%)	0.7 (−50%)	1.7 (−48%)	1.1 (−43%)

[a]Area ratios with respect to the external standard Z-Gly.

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■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and HPLC-UV chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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