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Triphosgene as highly efficient reagent for the solid-phase coupling of *N*-alkylated amino acids—total synthesis of cyclosporin O

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Abstract—A novel, highly efficient and racemization free coupling procedure for sterically hindered *N*-alkyl amino acids has been developed using triphosgene and a combination of diisopropylethyl amine (DIEA) and collidine at room temperature. Its efficiency was demonstrated by comparison with other coupling methods for *N*-alkylated amino acids and by solid-phase synthesis of the immunosuppressant cyclosporin O which was obtained in excellent purity and yield. © 2002 Elsevier Science Ltd. All rights reserved.

Coupling of *N*-alkylated amino acids is still a very challenging task, and a single method of choice for this problem has not yet emerged. That is especially true for syntheses on solid support, as the reactivity of resin bound secondary amines towards activated acids is even lower than in solution.[1] This is not only reflected in poor coupling yields, but also in a number of side reactions such as racemization or diketopiperazine formation which become more problematic as coupling rates decrease. The importance of an improved coupling procedure is due to the large number of potential synthetic targets containing *N*-alkylated peptide bonds, for example biologically active natural products such as cyclosporins, didemnins and dolastatins, or peptidomimetic structures such as backbone modified peptides.[2,3]

Some years ago, the superiority of HOAt and HOAt-based reagents over HOBt and its derivatives was shown for sterically hindered coupling reactions, and a combination of HATU and HOAt/DIC was successfully used for the preparation of cyclosporins on solid phase.[4] Coupling yields were in the range of 50–99% after double coupling reactions of up to 6 h each. In later attempts, symmetric anhydride couplings utilizing DCC as activating agent were employed. As before, multiple coupling reactions had to be done to achieve satisfactory coupling yields.[5] Better results, but with a considerably higher working expense, were achieved using site directed methylation.[6] Recently, Falb et al. used triphosgene (bis[trichloromethyl] carbonate, BTC) for the formation of *N*-alkyl amides, thus presenting a new approach to the problem.[7]

In a first evaluation study, we chose four different coupling methods to couple the amino acids Fmoc-Trp(Boc)-OH, Fmoc-Val-OH, Fmoc-Sar-OH and Fmoc-MeVal-OH onto resin bound MeVal-Phe. In

Table 1. Qualitative comparison of four methods for the coupling of different amino acids onto H-MeVal-Phe-TCP-resin

Coupling Method	Fmoc-Trp(Boc)-OH	Fmoc-Ile-OH	Fmoc-Sar-OH	Fmoc-MeVal-OH
HOAt	–	– –	+	– –
TFFH	– –	– –	–	–
DCC	– –	– –	+	– –
BTC	+	+	+	+

– –: chloranil test positive, –: chloranil test slightly positive, + chloranil test negative, indicating complete reaction.

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addition to HOAt/DIC and symmetric anhydride coupling using DCC, both of which have successfully been used in solid-phase cyclosporin syntheses, TFFH was tested as fourth reagent. TFFH was chosen to compare this in situ fluoride activation[8] with what Falb et al.[7] described as an in situ chloride activation in the case of triphosgene. Experimental details for HOAt-, TFFH- and DCC coupling reactions are summarized in Ref. 9, the triphosgene-coupling for this first study was carried out as reported by Falb et al.[7] Coupling efficiencies were qualitatively evaluated using chloranil test to check for free secondary amine on the resin.[10] All tripeptide products were cleaved from the resins and the chloranil test results verified by HPLC and MS. The results are summarized in Table 1.

The results show that the triphosgene method is by far superior to the other three methods. TFFH, which is one of the most powerful coupling reagents for α-branched amino acids,[11] is the least effective one in the case of *N*-methylated amino acids.

When we tried to use the original triphosgene method[7] for the construction of a longer peptide sequence, we were unsuccessful in isolating any cleavage product at the stage of the pentapeptide. This might be due to the fact that collidine alone is not basic enough to prevent the hydrochloric acid formed during the activation step from hydrolyzing the highly acid labile trityl ester resin linkage. When we tried to prevent this premature cleavage by using the less acid labile Wang resin, a different problem emerged. During Fmoc-deprotection of the dipeptide, quantitative diketopiperazine formation resulted. In the work by Falb et al., these problems did not arise because in this work triphosgene was used to prepare peptide amides on Rink amide resin.[7] We did, however, seek for a method to produce peptides with a free carboxy terminus. Another reason which prompted us to use the highly acid labile resin trityl chloride polystyrene resin (TCP resin),[12] is the well documented lability of *N*-alkylated peptides towards TFA.[13] To circumvent these problems, we developed a novel triphosgene-based coupling procedure using a special combination of bases for the preactivation and the coupling of the amino acids, respectively. The advantages of using a weak base for activation and a stronger base for coupling have been discussed extensively for the system HOAt/DIC.[14] In accordance with this work, we used collidine for preactivation of the carboxy component and DIEA for the pretreatment of the resin.

For a typical coupling reaction on 300 mg of dry resin with a loading of about 0.4 mmol/g of amino acid, 600 μl of DIEA/THF abs. (1:1) were added to the resin and the resin was swollen for 10 min. The Fmoc-amino acid (5 equiv.) was dissolved in a solution of BTC (1.65 equiv.) in dry THF (5 ml). After the solution had become clear, collidine (14 equiv.) was added, upon which a colorless precipitate formed. This suspension was gently shaken for about 1 min, after which it was added to the pretreated resin. The resin was shaken until a negative chloranil test showed the absence of free secondary amine functions (30 min–3 h).

This procedure allowed us to drive even such difficult coupling reactions as that of Fmoc-MeVal-OH onto H-MeVal-resin at room temperature in about 3 h to completion. Thus, heating of the reaction mixture was no longer necessary to obtain quantitative conversions. We compared this novel triphosgene coupling protocol with HATU and BEMT coupling methods.[15] The model reaction was the coupling of Fmoc-MeVal-OH to H-MeVal-MeIle-Sar-resin (Fig. 1). The thiazolium-based reagent BEMT was successfully used for the latest reported total synthesis of cyclosporin O.[16]

The same model reaction was used to check for racemization of our modified triphosgene coupling procedure. Couplings of racemic Fmoc-MeVal-OH, Fmoc-D-MeVal-OH and Fmoc-L-MeVal-OH were performed and the resulting tetrapeptides subjected to HPLC (Fig. 2). In the cases when enantiomerically pure Fmoc-D- or L-MeVal were used, no racemization product could be detected.

These results prompted us to proceed to a more interesting synthetic target. We chose cyclosporin O (CsO, Fig. 3), an immunosuppressive cycloundecapeptide containing seven *N*-methylated amino acids. We started off by synthesizing the tetrapeptide H-MeLeu-MeLeu-

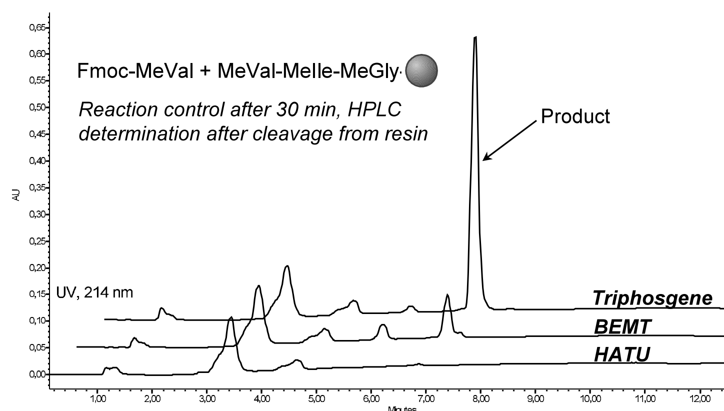

Figure 1. Comparison of HATU, BEMT and BTC mediated coupling. HPLCs of coupling products were recorded at 214 nm after 30 min reaction time and normalized to the peak of unreacted tripeptide.

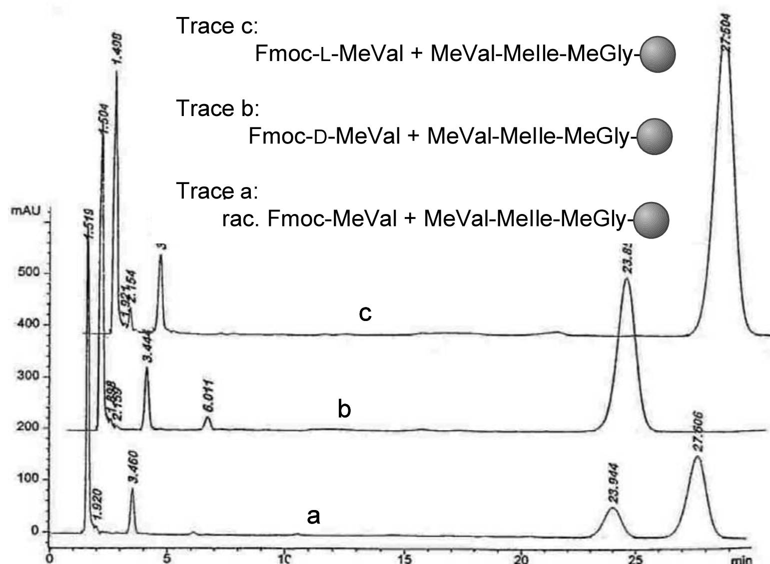

Figure 2. Comparison of HPLC-traces of tetrapeptides resulting from BTC-mediated coupling of (a) racemic Fmoc-MeVal, (b) Fmoc-D-MeVal and (c) Fmoc-L-MeVal. No racemization could be detected (214 nm).

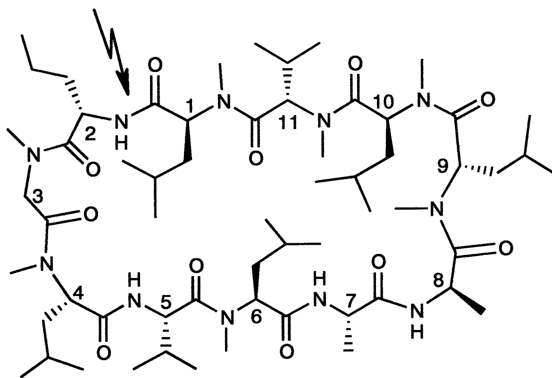

Figure 3. Cyclosporin O, cyclo(MeLeu-Nva-Sar-MeLeu-Val-MeLeu-Ala-D-Ala-MeLeu-MeLeu-MeVal). The arrow indicates the bond formed in the final cyclization step.

MeVal-MeLeu-OH [CsO(9-1)]. Being the longest sequence composed exclusively of *N*-methylated amino acids, this tetrapeptide is the most challenging substructure of CsO. Using the novel triphosgene method, we constructed this permethylated tetrapeptide in over 99% purity according to HPLC (214 nm). Only the first coupling reaction of MeVal to MeLeu had to be repeated, presumably because the sterical hindrance is higher in the proximity of the polymer backbone. The other coupling reactions were quantitative using a single coupling for 3 h or less at room temperature.

Over the whole course of the solid-phase peptide synthesis, four out of ten coupling reactions had to be repeated. As illustrated in Fig. 4, the BTC-mediated coupling of unmethylated amino acids was surprisingly more problematic than the coupling of *N*-methyl amino acids. The only exception to this rule was the quantitative BTC-mediated single coupling of Nva. We assume that the NH-proton of the urethane function might be responsible for a side reaction competing with the coupling reaction during BTC-activation. In these cases one additional coupling cycle using HOAt/DIC for 16 h drove the reaction to completion. Using this combined strategy of triphosgene and HOAt/DIC coupling reactions, the crude, deprotected linear undecapeptide was synthesized in only a few days with a purity of about 90% (Fig. 5).

Due to the high purity of the crude peptide and the fact that hexafluoroisopropanol could be used for the cleavage from TCP resin, the linear peptide could be cyclized after lyophilization without prior purification. The cyclization was carried out in DCM with HOAt, EDCI and DIEA (16 h) and proceeded with a crude yield of about 75%. After preparative HPLC, an overall yield of about 15% with respect to the first loading of the resin was calculated. Mass spectrometry and ^{1}H NMR proved the identity of the synthetic cyclosporin O with the natural product. The synthetic product was subjected to a chiral analysis by enantioselective GC–MS according to König et al.[17] The content of D-MeVal was found to be 0.7%, that of D-MeLeu 2.8%, thus showing negligible quantities of racemized compounds to be present in the product.

In summary, an optimized, reproducible and very efficient protocol for the solid-phase coupling of sterically hindered *N*-alkyl amino acids on highly acid labile trityl resin has been developed.[18] This novel method uses triphosgene as inexpensive activating reagent. It was shown to be superior to other methods for the coupling of *N*-alkyl amino acids, including activation with the expensive activation reagent HATU and the symmetrical anhydride method using DCC. Using the novel triphosgene method in combination with HOAt/DIC coupling reactions, the linear cyclosporin O undecapeptide was constructed in high yield and purity. Cyclization and purification led to cyclosporin O in an overall yield which lies well within the range of most of

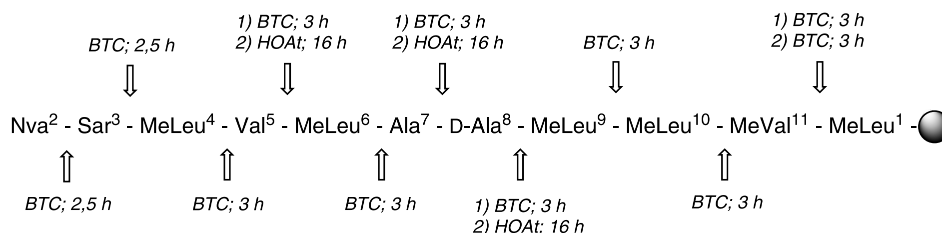

Figure 4. Graphical synthesis outline for the linear undecapeptide precursor of cyclosporin CsO(2-1).

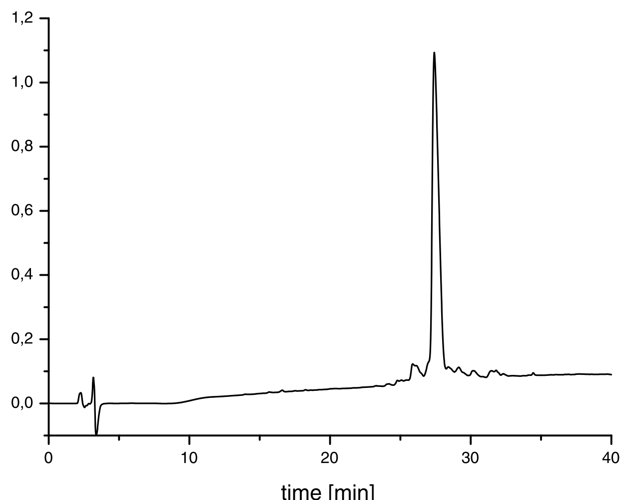

Figure 5. HPLC of crude, unprotected linear undecapeptide ($\lambda = 214$ nm).

the optimized liquid-phase syntheses reported for various cyclosporin analogs. In contrast to these liquid-phase syntheses, however, our approach on solid phase eliminates all intermediate purification procedures up to the final step of the synthesis, thereby reducing synthesis time and costs to a minimum.

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9. (a) HOAt-coupling: Fmoc-amino acid (3 equiv.) was dissolved in a solution of HOAt (3 equiv.) and DIEA (3 equiv.) in DMF. To this solution, DIC (3 equiv.) was added. After a 10 min preactivation period, the solution was added to the dipeptidyl resin. The suspension was shaken for 90 min.
(b) TFFH-coupling: Fmoc-amino acid (5 equiv.) was dissolved in a solution of TFFH (5 equiv.) and DIEA (10 equiv.) in DMF. The solution was added to the dipeptidyl resin and shaken for 90 min.
(c) DCC-coupling: Fmoc-amino acid (6 equiv.) was dissolved in a solution of DCC (3 equiv.) in DCM/DMF (3:1) and allowed to stand for 30 min. Dicyclohexyl urea was filtered off and the filtrate added to the dipeptidyl resin. The coupling time was 90 min.
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15. (a) HATU-coupling: Fmoc-amino acid (5 equiv.) was dissolved in DCM. HATU (5 equiv.) and DIEA (14 equiv.) were added. After a 10 min preactivation period, the solution was added to the dipeptidyl resin. The suspension was shaken for 30 min.
(b) BEMT-coupling: Fmoc-amino acid (5 equiv.) was dissolved in DCM. BEMT (5 equiv.) and DIEA (14 equiv.) were added. After a 10 min preactivation period, the solution was added to the dipeptidyl resin. The suspension was shaken for 30 min.
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