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Solid-Phase Synthesis of Peptide Esters Employing the Hydrazide Linker

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Many proteins involved in key processes of cell growth and differentiation embody ester groups at their C-terminus. For instance, the Ras-proteins and members of the Rab-proteins which are key players in biological signal transduction and the organization of vesicular transport terminate in a methyl ester;[1] furthermore, the Hedgehog protein that plays decisive roles in both embryogenesis and carcinogenesis carries cholesterol at its C-terminus.[2]

Since the ester groups influence the biological properties of these proteins, i.e., their anchoring to membranes and protein/protein interactions, for chemical biological studies an efficient access to such peptide esters is required.[3] In addition, peptide esters have been employed in peptide chemistry in a broader context also calling for the development of synthetic methodology.

In general, the synthesis of these esters can be achieved in solution using a condensation approach, i.e., by attachment of an esterified amino acid to a peptide chain. However, for subsequent biological investigations often series of peptide derivatives are required calling for the development of flexible solid phase techniques. Ideally, ester formation should be achieved in the cleavage step so that only the desired ester is released. On solid support, the synthesis of peptide methyl esters has been achieved using the Merrifield,[4] the 3-thiopropionic acid,[5] the oxime,[6] or the HMBA resin.[7] These linkers require basic conditions (tertiary base, methanol, respectively, sodium methoxide, methanol[5]) for the generation of the methyl ester by cleavage from the resin.

The purpose of this paper is to report on a new method for direct ester generation by cleavage from the solid support using the aryl hydrazide linker.[8] This linker group was originally introduced by Wieland et al.[9] and was used recently in the synthesis of cyclic peptides[10] and in the solid-phase synthesis of lipidated peptides.[11] It is cleaved by oxidation, e.g., with copper(II) acetate or with *N*-bromosuccinimde in the presence of a suitable nucleophile (Scheme 1), and orthogonally stable to established urethane blocking groups (Boc, Fmoc, Aloc). The target peptides are released from the resin without racemiziation,[10] and the cleavage conditions are compatible with amino acids embodying oxidation-labile functional groups, e.g., methionine.[11] To determine whether direct ester formation by cleavage from the solid support is generally applicable, we investigated a variety of different alcohols as nucleophiles. The main advantage of the described approach is that the elongation of the peptide chain can be carried out by means of standard methodologies after attachment of the first amino acid via its carboxy function. After construction of the peptide, the cleavage is carried out under mild oxidative conditions that allow easy formation of the desired ester.

In the course of this process, the hydrazide **1** is oxidized to the corresponding acyldiazene **2** (Scheme 1), which is then trapped by an added nucleophile releasing the corresponding conjugates from the solid support. Thus, if different alcohols are used different esters **3** are obtained.

The Fmoc group was cleaved from commercially available 2-Fmoc-hydrazinobenzoyl AM resin **1**[12] (loading 0.67 mmol g^{-1}) using 20% piperidine in DMF (Scheme 2). Subsequently, tripeptides **4** were built up using standard Boc methodology. To prevent acylation of the hydrazide linker nitrogen atoms during the capping step, the sterically hindered pivalic acid anhydride was chosen as capping reagent. After coupling of the last amino acid (Fmoc protected), the resulting loading was determined,[13] allowing for a fairly accurate estimation of the yield for the final cleavage reaction.

The oxidative cleavage was carried out either by using a one-step process (method A) with copper(II) acetate in the presence of a suitable nucleophile or in a two-step process (method B) using *N*-bromosuccinimide as oxidizing agent[14] and subsequent addition of the nucleophile after removal of surplus reagent. As nucleophiles a variety of different alcohols were used to obtain different peptide esters **6** (Table 1). To prevent the formation of the free peptide acid by aqueous hydrolysis of the acyldiazene **5**, it is essential to remove traces of water from the nucleophiles.

Table 1 demonstrates that this methodology offers an efficient way for the synthesis of peptide esters. Both esters of primary and secondary alcohols are readily

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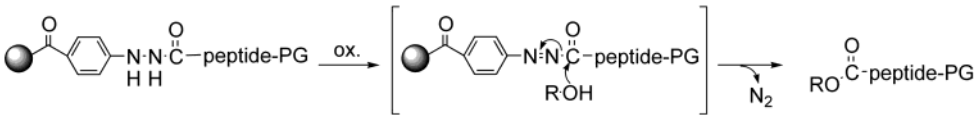
SCHEME 1. Principle of Peptide Ester Formation Employing the Oxidation Labile Hydrazide Linker



SCHEME 2. Peptide Synthesis and Oxidative Release of Peptide Esters from the Hydrazide Linker

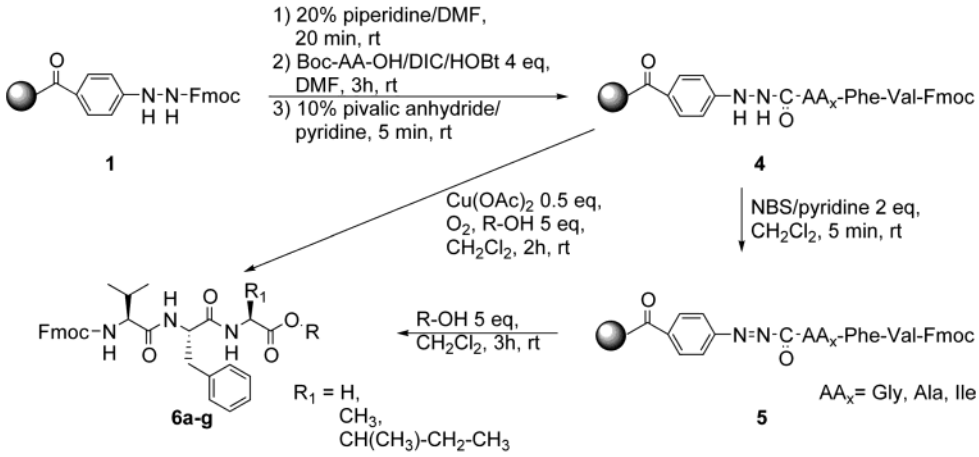


TABLE 1. Formation of Peptide Esters 6

no.	nucleophile	peptide	oxidation A[a] (%)	oxidation B[a] (%)
6a	methanol	Fmoc-Val-Phe-Gly-OMe	55	64
6b	allyl alcohol	Fmoc-Val-Phe-Gly-OAll	58	*c*
6c	2-propanol	Fmoc-Val-Phe-Gly-O*i*Pr	43	49
6d	1-heptanol	Fmoc-Val-Phe-Ile-OHep	48	52
6e	benzyl alcohol	Fmoc-Val-Phe-Ala-OBzl	34	44
6f	cyclohexanol	Fmoc-Val-Phe-Gly-O*c*Hex	acid[b]	43
6g	1-decalol	Fmoc-Val-Phe-Gly-ODec	acid[b]	39

[a] Yields based on loading determined after coupling of the last amino acid. [b] Traces of acid were detected by HPLC-MS. [c] Not investigated.

formed. The alcohols can incorporate further functionalities such as a double bond. Most notably, the desired compounds are obtained by this technique with hardly any byproduct formation. This is particularly true for the two-step process. The acyldiazene formed after the oxidation with NBS is stable, and all contaminants and reagents can simply be removed by washing and filtration. If the oxidation is carried out with copper the heavy metal is readily removed by an operationally simple solid-phase extraction (SPE) cartridge. The yield of the cleavage step correlates directly with the steric demand of the nucleophile: primary alcohols are good nucleophiles and allow cleavage in yields of ca. 50−60%. These yields represent the cleavage step only and in the case of effective peptide-coupling they correspond to the overall yield. For secondary alcohols and for sterically demanding C-terminal amino acids the yield is somewhat lower but still in the preparatively useful range. For secondary nucleophiles, the two-step process employing oxidation with NBS yields superior results.

The successful use of allyl alcohol, heptanol, and benzyl alcohol deserves particular attention. These esters are among the most efficient and widely used C-terminal protecting groups employed in solution-phase peptide chemistry as well as in various other fields of organic synthesis. Thus, the benzyl ester and the allyl ester are standard protecting groups that have found widespread application.[15] In particular, the palladium(0)-labile allyl esters are successfully applied in the generation of glycopeptides and lipopeptides.[16,17] The heptyl ester is an enzyme-labile protecting group and was employed in the synthesis of phosphopeptides.[18]

Furthermore, we would like to point out that the hydrazide linker is compatible to a variety of different transformations and reaction conditions and that it has been applied successfully in different solid-phase sequences leading to biologically active nonpeptidic compounds.[19−23] Thus, the ability to generate different ester groups in the final cleavage step adds to this synthetic diversity and opens up a further opportunity for combinatorial chemistry by potentially giving access to ester libraries.

Experimental Section

Peptide Synthesis. After removal of the Fmoc group, coupling of the amino acids was achieved by shaking the resin with a solution of 4 equiv of Boc-amino acid, 4 equiv of diisopropylcarbodiimide, and 4.4 equiv of 1-hydroxybenzotriazole

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in DMF for 3 h at room temperature. The capping step was carried out by treating the resin with a 10% solution of pivalic anhydride in pyridine for 5 min. For removal of the Boc-protecting groups, the resin was treated twice with a 1:1 mixture of trifluoroacetic acid and dichloromethane.

Oxidation. Method A. In a typical procedure, 200 mg of the resin was added to a solution of 0.5 equiv (relative to the loading estimated after last coupling) of $Cu(OAc)_2$, 10 equiv of pyridine, and 5 equiv of the corresponding nucleophile in dry dichloromethane. The resulting suspension was shaken for 2 h at room temperature while oxygen was bubbled through. After filtration, the resin was washed five times with dichloromethane, and the combined filtrates were concentrated in vacuo. The copper salts were removed by using a SPE-cartridge (Supelco) and elution of the peptide ester with dichloromethane.

Method B. The resin (200 mg) was treated with a solution of *N*-bromosuccinimide and pyridine (2 equiv each) in 5 mL of dry dichloromethane for 5 min, drained, and washed three times each with dry dichloromethane and dry tetrahydrofuran. After drying, the mixture was shaken with a solution of 5 equiv of the corresponding nucleophile in dry dichloromethane for 4 h at room temperature. The resin was filtered and washed five times with dichloromethane, and the product was purified using preparative HPLC (125−21 C4 column; Macherey-Nagel) as stationary phase and a linear acetonitrile/water gradient (10−100%, 10 min), 0.1% trifluoroacetic acid; the product was detected at $\lambda = 210$ nm).

Fmoc-Val-Phe-Gly-OMe (6a): colorless crystals; mp = 191 °C; $[\alpha]^{20}_D = -40.6$ (*c* 0.17, $CHCl_3$/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, ^3J = 7.9 Hz, 2H), 7.58 (d, ^3J = 7.6 Hz, 2H), 7.41−7.37 (m, 2H), 7.33−7.28 (m, 2H), 7.23−7.12 (m, 5H), 4.70 (t, ^3J = 7.6 Hz), 4.45−4.32 (m, 2H), 4.19 (t, ^3J = 7.0 Hz), 4.07−4.02 (m, 1H), 3.86−3.82 (m, 2H), 3.68 (s, 3H), 3.19 (dd, ^2J = 6.5 Hz, ^3J = 13.9 Hz, 1H), 2.94 (dd, ^2J = 8.3 Hz, ^3J = 13.9 Hz, 1H), 2.03−1.98 (m, 1H), 0.78 ppm (d, ^3J = 6.7 Hz, 6H); FAB-MS (*m*/*z*) calcd 558.2604 $[M + H]^+$, found 558.2593 (in 3-NBA); HPLC t_R = 7.96 min (purity: 96%).

Fmoc-Val-Phe-Gly-OAll (6b): colorless crystals; mp = 189 °C; $[\alpha]^{20}_D = -28.9$ (*c* 0.19, $CHCl_3$/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, ^3J = 7.4 Hz), 7.56 (d, ^3J = 7.2 Hz), 7.39−7.35 (m, 2H), 7.30−7.28 (m, 2H), 7.20−7.10 (m, 5H), 5.88−5.78 (m, 1H), 5.28−5.19 (m, 2H), 4.69 (t, ^3J = 7.6 Hz, 1H), 4.55 (d, ^3J = 5.5 Hz, 2H), 4.44−4.29 (m, 2H), 4.17 (t, ^3J = 6.6 Hz, 1H), 4.07−4.01 (m, 1H), 3.88−3.83 (m, 2H), 3.18 (dd, ^2J = 6 Hz, ^3J = 14 Hz), 2.91 (dd, ^2J = 8 Hz, ^3J = 14 Hz), 2.01−1.94 (m, 1H), 0.76 ppm (d, ^3J = 6.6 Hz, 6H); FAB-MS (*m*/*z*) calcd 584.2761 $[M + H]^+$, found 584.2758 (in 3-NBA); HPLC t_R = 7.96 min (purity: 91%).

Fmoc-Val-Phe-Gly-O*i*Pr (6c): colorless crystals; mp = 186 °C; $[\alpha]^{20}_D = -31.1$ (*c* 0.14, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3$) δ = 7.73 (d, ^3J = 7.6 Hz, 2H), 7.56 (d, ^3J = 7.4 Hz, 2H), 7.38−7.35 (m, 2H), 7.29−7.26 (m, 2H), 7.20−7.09 (m, 5H), 4.99−4.92 (m, 1H) 4.67 (t, ^3J = 7.4 Hz, 1H), 4.43−4.27 (m, 2H), 4.16 (t, ^3J = 6.8 Hz, 1H), 3.98−3.94 (m, 1H), 3.83−3.75 (m, 2H), 3.17 (dd, ^2J = 6.4 Hz, ^3J = 13.6 Hz, 1H), 2.92 (dd, ^2J = 8.1 Hz, ^3J = 13.6 Hz), 1.99−1.93 (m, 1H), 1.18 (dd, ^2J = 6.6 Hz, ^3J = 9 Hz, 6H), 0.75 ppm (d, ^3J = 6.8 Hz, 6H); FAB-MS (*m*/*z*) calcd 586.2917 $[M + H]^+$, found 586.2891 (in 3-NBA); HPLC t_R = 8.06 min (purity: 98%).

Fmoc-Val-Phe-Ile-OHep (6d): film; $[\alpha]^{20}_D = -25.2$ (*c* 0.33, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3$) δ 7.74 (d, ^3J = 7.5 Hz, 2H), 7.57 (d, ^3J = 7.4 Hz, 2H), 7.39−7.36 (m, 2H), 7.31−7.27 (m, 2H), 7.19−7.07 (m, 5H), 4.63−4.60 (m, 1H), 4.44−4.37 (m, 2H), 4.31−4.27 (m, 1H), 4.18 (t, ^3J = 7.0 Hz, 1H), 4.04 (t, ^3J = 6.7 Hz, 2H), 3.87 (d, ^3J = 6.8 Hz, 1H), 3.07 (dd, ^2J = 6.6 Hz, ^3J = 13.8 Hz), 2.91 (dd, ^2J = 7.9 Hz, ^3J = 13.8 Hz), 1.98−1.93 (m, 1H), 1.84−1.74 (m, 1H), 1.60−1.55 (m, 2H), 1.25−1.19 (m, 9H), 1.18−1.06 (m, 1H), 0.86−0.80 ppm (m, 15H); FAB-MS (*m*/*z*) calcd 720.3989 $[M + Na]^+$, found 720.4007 (in 3-NBA); HPLC t_R = 9.18 min (purity: 95%).

Fmoc-Val-Phe-Ala-OBzl (6e): film; $[\alpha]^{20}_D = -26.8$ (*c* 0.38, $CHCl_3$/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.69 (d, ^3J = 7.5 Hz), 7.53−7.51 (m, 2H), 7.34−7.31 (m, 2H), 7.29−7.27 (m, 2H), 7.24−7.22 (m, 5H), 7.13−7.03 (m, 5H), 5.06 (s, 2H), 4.57−4.52 (m, 1H), 4.44−4.35 (m, 2H), 4.26−4.22 (m, 1H), 4.12 (t, ^3J = 6.7 Hz, 1H), 3.80 (d, ^3J = 6.6 Hz, 1H), 3.02 (dd, ^2J = 6.0 Hz, ^3J = 14.0 Hz, 1H), 2.84 (dd, ^2J = 8.0 Hz, ^3J = 14.0 Hz, 1H), 1.94−1.88 (m, 1H), 1.28 (d, ^3J = 7.0 Hz, 3H), 0.75 ppm (d, ^3J = 6.6 Hz, 6H); FAB-MS (*m*/*z*) calcd 648.3074 $[M + H]^+$, found 648.3084 (in 3-NBA); HPLC t_R = 7.27 min (purity 96%).

Fmoc-Val-Phe-Gly-O*c*-Hex (6f): colorless crystals; mp = 180 °C; $[\alpha]^{20}_D = -25.0$ (*c* 0.72, $CHCl_3$/MeOH 1:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, ^3J = 7.6 Hz, 2H), 7.56 (d, ^3J = 7.4 Hz, 2H), 7.39−7.35 (m, 2H), 7.30−7.28 (m, 2H), 7.20−7.10 (m, 5H), 4.74−4.67 (m, 2H), 4.43−4.28 (m, 2H), 4.17 (t, ^3J = 6.8 Hz, 1H), 4.00−3.96 (m, 1H), 3.85−3.77 (m, 2H), 3.18 (dd, ^2J = 6.2 Hz, ^3J = 14 Hz, 1H), 2.91 (dd, ^2J = 8.4 Hz, ^3J = 14 Hz, 1H), 2.01−1.92 (m, 1H), 1.74−1.63 (m, 4H), 1.46−1.19 (m, 6H), 0.76 ppm (d, ^3J = 6.7 Hz, 6H); FAB-MS (*m*/*z*) calcd 626.3230 $[M + H]^+$, found 626.3221 (in 3-NBA); HPLC t_R = 8.54 min (purity: 97%).

Fmoc-Val-Phe-Gly-ODec (6g): colorless crystals; mp = 176 °C; $[\alpha]^{20}_D = -27.5$ (*c* 0.24, $CHCl_3$/MeOH 1:1); ^{1}H NMR(400 MHz, $CDCl_3$) δ = 7.72 (d, ^3J = 7.4 Hz, 2H), 7.55 (d, ^3J = 7.2 Hz, 2H), 7.37−7.34 (m, 2H), 7.29−7.25 (m, 2H), 7.19−7.11 (m, 5H), 4.77−4.64 (m, 2H), 4.42−4.26 (m, 2H), 4.16 (t, ^3J = 6.4 Hz, 1H), 3.98−3.93 (m, 1H), 3.82−3.76 (m, 2H), 3.13 (dd, ^2J = 6.3 Hz, ^3J = 13.8 Hz, 1H), 2.88 (dd, ^2J = 8.6 Hz, ^3J = 13.8 Hz, 1H), 1.98−1.89 (m, 2H), 1.73−1.62 (m, 3H),1.57−1.11 (m, 14H), 0.75 ppm (d, ^3J = 6.8 Hz, 6H); FAB-MS (*m*/*z*) calcd 680.3700 $[M + H]^+$, found 680.3713 (in 3-NBA); HPLC t_R = 9.18 min (purity 95%).

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