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Stereoselective C-terminal peptide elongation from Chan–Lam–Evans reaction generated isopropenyl esters†

Luuk Steemers and Jan H. van Maarseveen D*

C-Terminal dipeptide isopropenyl esters were synthesised by a Cu(II)-mediated Chan–Lam–Evans enol esterification of peptide carboxylic acids and isoprenyl boroxyline. These shelf stable peptide esters could be coupled stereoselectively with a variety of amino acid and dipeptide nucleophiles in high yield and purity in the presence of pyrazole/DBU as the catalyst.

In current pharmaceutic research peptides play an increasingly important role.1 As a result, in recent years several peptides and analogs thereof were registered as drugs.2 By using both solid and solution phase techniques peptides are synthesized in the C → N direction exclusively. In other words, the N-terminus is elongated by consecutive coupling of single N-protected amino acids. Commonly employed carbonyl group activating reagents, for example uronium, phosphonium and carbodiimide based, form a highly reactive intermediate, that is usually transformed in situ into less reactive HOBt or HOAt esters to avoid racemisation.3 The mesomerically stabilised and, as compared to an amide group, less nucleophilic N-carbamate protective group suppresses racemisation of the stereogenic center by hampering enolisation or oxazolone formation. Especially for peptide fragment couplings or peptide cyclisation C-terminal peptide activation is required. However, coupling of two peptide fragments or cyclisation with traditional coupling reagents inevitably leads to epimerisation of the α-carbon of the C-terminally carboxyl-activated residue. As a result, in current ligation or cyclisation strategies, usually a C-terminal glycine is chosen as the site of carboxyl activation.4 In stark contrast, both ribosomally and non-ribosomally synthesised peptides are elongated in the N → C direction, via aminolysis of the growing peptide chain of the activated C-terminus. Nature uses very mild carboxyl group activation strategies, such as the connecting ester bond between the amino acid and tRNA, or as a thioester while linked at the peptidyl carrier protein in the case of non-ribosomal peptide synthesis. Aminolysis of the rather unreactive secondary ester at the tRNA occurs due to the proximity of the incoming amine nucleophile to this ester, induced by the ribosome, underscoring the power of pre-organisation for certain chemical transformations.

Synthetic strategies employing elongation in the N → C direction are scarce. Katritzky has shown that peptide benzo-triazole esters may be prepared in the cold using SOCl2/HOBt and subsequently coupled to other peptide fragments.5 More recently, the Bode group has shown that unprotected peptides bearing a C-terminal keto acid ligate with peptides modified with a hydroxylamine at the N-terminus.6 Another viable method used for fragment couplings is the synthesis of peptides that deliver a stable C-terminal ester serving as a substrate for chemoenzymatic coupling.7 In the search for catalytic carboxyl group activation methods to substitute the traditional stoichiometric coupling reagents we have reported earlier the use of mildly activated 4-methylsulfonylphenyl esters, obtained via a Cu(II)-catalysed Chan–Lam–Evans (CLE) reaction and subsequent oxidation, as suitable substrates for epimerisation-free coupling (Scheme 1).9

† Electronic supplementary information (ESI) available: Detailed experimental procedures and copies of the 1H- and 13C-NMR spectra and chiral HPLC traces. See DOI: 10.1039/c8ob02102c

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It was found that the CLE-type aryl esterification of peptides proceeded with complete stereoretention. However, to obtain high de’s in the aminolysis reaction the amine had to be added slowly to avoid too basic conditions. Recently, we disclosed the efficient enol esterification of carboxylic acids via the CLE reaction and demonstrated the compatibility with all proteogenic amino acids, except methionine. Moreover, no racemisation of the C-terminus was observed during the CLE-type enol esterification of amino acids. This prompted us to study the possibility of stereoselective elongation of peptides at the C-terminus employing isopropenyl esters as mild activating groups. As compared to 4-methylsulfonylphenyl esters, isopropenyl esters are less reactive and only react slowly in direct aminolysis reactions. Recently, the group of Birman reported anionic azoles as efficient catalysts for the aminolysis of both phenyl and isopropenyl esters. Remarkably, in a landmark paper that was far ahead of its time, the group of Beyerman reported back in the 1960s the use of neutral azoles as additives for the stereoselective aminolysis of peptide vinyl esters that were made via Pd-catalysis. Although vinyl esters can be efficiently synthesized via the CLE reaction from commercially available trivinylboroxine but also by Pd(II) or Hg(II) cata-

Table 1 Transformation of the dipeptide carboxylic acids into their isopropenyl esters using the CLE-reaction

| Starting dipeptide | Starting peptide acid product | Yield (%) | de (%) |
|--------------------|-------------------------------|-----------|--------|
| 1a                 | Boc-Phe-Ala-OH                | 92        | nd     |
| 1b                 | Boc-Phe-D-Ala-OH              | 91        | nd     |
| 1c                 | Boc-Phe-Phe-OH                | 90        | >99    |
| 1d                 | Boc-Phe-D-Phe-OH              | 97        | >99    |
| 1e                 | Boc-Phe-Val-OH                | 80        | nd     |
| 1f                 | Boc-Phe-D-Val-OH              | 91        | nd     |

The optimisation studies started using CH2Cl2 as solvent and by adding a slight excess of 1,2,4-triazole as a catalyst and 10 mol% DBU as the base to generate the active anionic azolate species. Although the aminolysis reaction was clean and did not require further purification besides a simple acidic washing step, only a moderate de of 69% was found (Table 2, entry 1). By using pyrazole as the catalyst, a similar outcome of the reaction was found (entry 2). A significant improvement of the de was achieved by increasing the amount of azole to five equiv. (entry 3). By adding two equiv. of the amine nucleophile (entry 4) the de went further up to 89%. Switching to toluene as a more apolar solvent helped to improve the de even further to 97% (entry 5 vs. 1 and entry 6 vs. 4). Also in this case pyrazole performed slightly better than 1,2,4-triazole (entry 7 vs. 6). Raising the amount of amine or using an even more apolar solvent did not lead to a further increase of the de (entries 8 and 9). Also 1,2,3-triazole and benzotriazole were screened as catalysts, however these reactions either did not reach full conversion, had lower de and/or produced unidentified byproducts (not shown).

The proposed reaction mechanism for the pyrazole-catalysed aminolysis reaction starts by deprotonation of pyrazole I by DBU to form the highly nucleophilic azole anion II (Scheme 2). Nucleophilic attack of the anionic nitrogen at the isopropenyl ester provides acylpyrazole III, thereby irreversibly releasing acetone and regenerating DBU. The incoming amine nucleophile can coordinate to the pyrazole 2-nitrogen atom via H-bonding forming complex IV showing a favourable 5-mem-

Table 2 Optimisation of the aminolysis reaction

| Entry | Solvent | Azole (equiv.) | Equiv. H-Ala-OrBu | de (%) |
|-------|---------|----------------|-------------------|--------|
| 1     | CH2Cl2  | 1,2,4-Triazole (1.2) | 1.2              | 69     |
| 2     | CH2Cl2  | Pyrazole (1.2)     | 1.2              | 72     |
| 3     | CH2Cl2  | 1,2,4-Triazole (5) | 1.2              | 79     |
| 4     | CH2Cl2  | 1,2,4-Triazole (5) | 2                | 89     |
| 5     | PhCH3   | 1,2,4-Triazole (1.2) | 1.2             | 84     |
| 6     | PhCH3   | 1,2,4-Triazole (5) | 2                | 95     |
| 7     | PhCH3   | Pyrazole (5)       | 2                | 97     |
| 8     | PhCH3   | Pyrazole (5)       | 4                | 96     |
| 9     | PhH     | Pyrazole (5)       | 2                | 95     |

* Determined by chiral HPLC.
gave either incomplete or no conversion at all at room temperature. For Gly-OrBu or Ala-OrBu as nucleophiles, heating to 50°C was sufficient to complete the reaction, giving tripeptides 3q-t in excellent yields and diastereomeric excess. However, in the case of using Phe-OrBu or Val-OrBu as nucleophiles heating to 80°C was required to ensure completion of the reaction. Although the yields of products 3v-x were still very good, we had to accept that the de’s showed a small but significant drop. Presumably this is caused by the longer living epimerisation-prone acylpyrazole intermediate III (see Scheme 2).

To mimic more closely a true peptide ligation reaction, the dipeptide isopropenyl esters 2a-f were subjected to pyrazole-catalysed aminolysis by reaction with dipeptide H-Phe-Phe-OrBu as the nucleophile.18 By application of the optimised conditions, Boc-Phe-Ala-Phe-OrBu (4a) and Boc-Phe-Ala-Phe-OrBu (4b) were isolated in yields of 68% and 79%, respectively. This drop of the yield as compared to using amino acid esters as the nucleophile was caused by the fact that the excess of apolar H-Phe-Phe-OrBu could not be removed by simply acidic washing. Moreover, chromatographic purification was unsuccessful due to the similar Rf values of H-Phe-Phe-OrBu and the tetrapeptide products. The problem was overcome by reacting the crude mixture with Boc2O to transform the excess of unreacted nucleophile into Boc-Phe-Phe-OrBu, which could be separated via column chromatography.

### Table 3 Synthesis of tripeptides

| Starting dipeptide | Tripeptide product         | Yield (%) | de (%) |
|--------------------|----------------------------|-----------|--------|
| 2a                 | Boc-Phe-Ala-Gly-OrBu       | 96        | 97     |
| 2b                 | Boc-Phe-D-Ala-Gly-OrBu     | 98        | 98     |
| 2a                 | Boc-Phe-Ala-OrBu           | 99        | 99     |
| 2b                 | Boc-Phe-D-Ala-OrBu         | 93        | 93     |
| 2a                 | Boc-Phe-Ala-Phe-OrBu       | 95        | 95     |
| 2b                 | Boc-Phe-D-Ala-Phe-OrBu     | 92        | 92     |
| 2a                 | Boc-Phe-Ala-Val-OrBu       | 98        | 98     |
| 2b                 | Boc-Phe-D-Ala-Val-OrBu     | 95        | 95     |
| 2c                 | Boc-Phe-Phe-Gly-OrBu       | 94        | 94     |
| 2d                 | Boc-Phe-Phe-Val-OrBu       | 90        | 90     |
| 2e                 | Boc-Phe-Phe-Val-OrBu       | 90        | 90     |
| 2f                 | Boc-Phe-Phe-Val-OrBu       | 97        | 97     |
| 2g                 | Boc-Phe-Phe-Val-OrBu       | 97        | 97     |
| 2h                 | Boc-Phe-Phe-Val-OrBu       | 98        | 98     |
| 2i                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2j                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2k                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2l                 | Boc-Phe-Phe-Val-OrBu       | 98        | 98     |
| 2m                 | Boc-Phe-Phe-Val-OrBu       | 97        | 97     |
| 2n                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2o                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2p                 | Boc-Phe-Phe-Val-OrBu       | 98        | 98     |
| 2q                 | Boc-Phe-Phe-Val-OrBu       | 97        | 97     |
| 2r                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2s                 | Boc-Phe-Phe-Val-OrBu       | 98        | 98     |
| 2t                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2u                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2v                 | Boc-Phe-Phe-Val-OrBu       | 98        | 98     |
| 2w                 | Boc-Phe-Phe-Val-OrBu       | 99        | 99     |
| 2x                 | Boc-Phe-Phe-Val-OrBu       | 97        | 97     |

* Determined by chiral HPLC. b Reaction run at 50°C. c Reaction run at 80°C.
and Boc-Phe-D-Phe-Phe-Phe-O

case the Phe peptide series similar results were observed, however in this
Future work will focus on applications in peptide cyclizations

| Starting dipeptide | Tetrapeptide product | Yield (%) | de (%) |
|-------------------|----------------------|-----------|--------|
| 2a                | Boc-Phe-Ala-Phe-Phe-OrBu | 4a | 68 | 99 |
| 2b                | Boc-Phe-D-Ala-Phe-Phe-OrBu | 4b | 79 | 99 |
| 2c                | Boc-Phe-Phe-Phe-Phe-OrBu | 4c | 69 | 93 |
| 2d                | Boc-Phe-D-Phe-Phe-Phe-OrBu | 4d | 69 | 93 |
| 2e                | Boc-Phe-Val-Phe-Phe-OrBu | 4e | 47 | 95 |
| 2f                | Boc-Phe-D-Val-Phe-Phe-OrBu | 4f | 52 | 97 |

* Reaction run at 70 °C.

Robust, high yielding and scalable methodology was developed to transform the C-terminal carboxylic acid of dipeptides with complete stereoretention into isopropenyl esters via a CLE reaction. Further elongation into tri- and tetrapeptides was carried out via a mild pyrazole catalysed aminolysis reaction liberating acetone as an inert side product. For the tetrapeptide series, high yields of crude product were obtained, which required no further purification. Excellent de’s (>96%) were observed for almost all tested tripeptides. Only hindered couplings, such as for making the connecting peptide bond within Val-Phe and Val-Val showed a small drop in de. For the tetrapeptide series similar results were observed, however in this case the Phe–Phe coupling also showed a small drop in de. Future work will focus on applications in peptide cyclizations and the ligation of larger, more diverse peptide fragments.

Conclusions

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Conflicts of interest

There are no conflicts to declare.

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