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Rapid and high-yield solution-phase synthesis of DOTA-Tyr^3-octreotide and DOTA-Tyr^3-octreotate using unprotected DOTA

Margret Schottelius,* Markus Schwaiger and Hans-Jürgen Wester

Nuklearmedizinische Klinik und Poliklinik, Klinikum rechts der Isar, Technische Universität München, Ismaninger Strasse 22, D-81675 München, Germany

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Abstract—An improved method for the solution-phase derivatization of Tyr^3-Lys^5(Dde)-octreotide (TOC(Dde)) and Tyr^3-Lys^5(Dde)-octreotate (TATE(Dde)) with the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-*N′*,*N″*,*N‴*,*N⁗*-tetra-acetic acid) has been developed. The fully protected parent peptides were assembled via solid-phase peptide synthesis (SPPS) using Fmoc-strategy. After cleavage from the solid support, disulfide bond formation was carried out using H_2O_2. Both TOC(Dde) and TATE(Dde) were successfully coupled with DOTA in the presence of NHS, EDCI and DIPEA in a water/DMF solvent system. Yields of the coupling reaction were >98% within only 2 h with no detectable formation of sideproducts. This method for the preparation of DOTATOC, DOTATATE and other DOTA-peptide conjugates is therefore a rapid and economic alternative to the currently used methods.

The application of radiolabelled octreotide analogs for peptide receptor mediated radionuclide therapy (PRRT) of somatostatin receptor overexpressing human tumors is an emerging field in nuclear medicine. In particular, radiometal-chelate containing octreotide derivatives such as [^{90}Y]DOTATOC[1] ([^{90}Y]DOTA-D-Phe^1-Tyr^3-octreotide) or [^{177}Lu]DOTATATE[2] ([^{177}Lu]-DOTA-D-Phe^1-Tyr^3-octreotate) have shown high therapeutic efficacy in preclinical and clinical studies.[3–5] Based on these encouraging results, clinical application of radiometallated DOTA-analogs of octreotide for internal radiotherapy as well as the development of other DOTA-conjugated neuropeptide analogs[6–9] is steadily increasing. This necessitates in turn the development of high-yield syntheses for the preparation of DOTA-coupled radiolabelling precursors. For example, in the case of DOTATOC and DOTATATE, depending on the activity applied and the radionuclide used, 50–500 μg (^{90}Y ≪ ^{177}Lu) of DOTA-conjugated peptide are needed per patient for the preparation of therapeutic doses.[10]

Thus, different chemical methodologies have been investigated to optimize the synthesis of DOTA-conjugated peptides and in particular of DOTATOC: (***A***) coupling of unprotected DOTA to Lys^5(Boc)-Tyr^3-octreotide in aqueous DMF solution,[11] (***B***) on-resin derivatization of fully protected Tyr^3-octreotide with DOTA tri(tetrabutylammonium hydroxide) salt,[12] (***C***) total solid-phase synthesis of the DOTA chelator on peptidyl resin,[13] and (***D***) solution-phase coupling with monoreactive DOTA.[14–17]

Currently, the preferred synthesis route is ***D***, since it allows selective peptide derivatization in high yields without the formation of doubly substituted DOTA-sideproducts. In contrast to method ***A***, however, ***D*** has the disadvantage of requiring costly starting materials and/or a multistep synthesis of the monoreactive DOTA analog.[14] Furthermore, yields for the coupling step were reported to be only 60%, when equimolar amounts of DOTA-tris-*tert*-butyl ester[18] and peptide were used.[14] In addition, problems were encountered in our laboratory during the final cleavage of the DOTA *tert*-butyl protecting groups with 95% trifluoroacetic acid (TFA), resulting in either incomplete deprotection or peptide degradation.

To circumvent these drawbacks, we have developed an improved solution-phase method based on method ***A*** (Scheme 1) for medium scale (100–500 mg of peptide) synthesis of DOTA-coupled octreotide and octreotate.

* Corresponding author. Tel.: +49-89-4140-4554; fax: +49-89-4140-4897; e-mail: m.schottelius@lrz.tum.de

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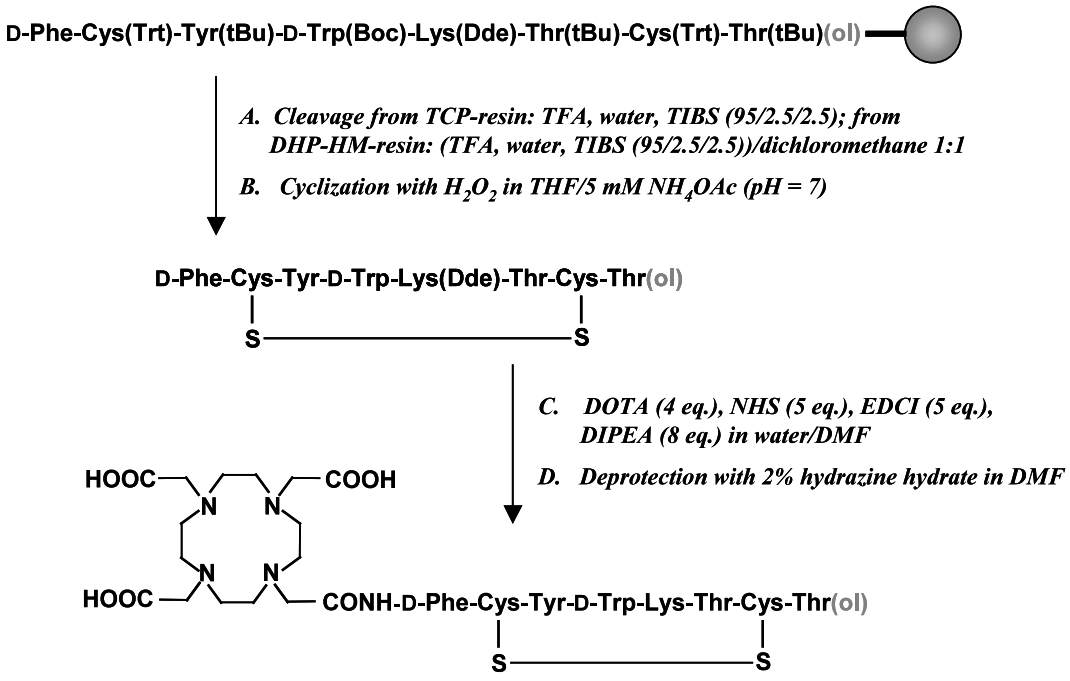

Scheme 1. Reaction sequence for the solution-phase synthesis of DOTATOC (C-terminal Thr(ol) anchored to DHP-HM-resin) and DOTATATE (C-terminal Thr anchored to TCP-resin) after SPPS of the parent peptides.

For solid-phase peptide synthesis (SPPS) of Lys[5](Dde)-Tyr[3]-octreotide (TOC(Dde)), Fmoc-Thr(*t*Bu)-ol was anchored to DHP-HM resin (3,4-dihydro-2*H*-pyran-2-ylmethoxymethyl polystyrene) in the presence of catalytic amounts of pyridinium *p*-toluenesufonate in 1,2-dichloroethane at 80°C for 16–18 h. SPPS of Lys[5](Dde)-Tyr[3]-octreotate (TATE(Dde)) was carried out using TCP (tritylchloride polystyrene) resin. Coupling of Fmoc-Thr(*t*Bu)-OH to the solid support was performed in dry dichloromethane in the presence of an excess of DIPEA (*N*-ethyldiisopropylamine). The linear octapeptides D-Phe-Cys(Trt)-Tyr(*t*Bu)-D-Trp(Boc)-Lys(Dde)-Thr(*t*Bu)-Cys(Trt)-Thr(*t*Bu)/Thr(*t*Bu)*ol* were assembled in NMP (*N*-methylpyrrolidon) using a standard Fmoc-protocol, i.e. 1.5 equiv. of Fmoc-amino acid, HOBt (*N*-hydroxybenzotriazole) and TBTU (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate), respectively, and 3.5–4 equiv. of DIPEA. After removal of the N-terminal Fmoc-protecting group, TATE(Dde) was cleaved from the solid support using a mixture of TFA (95%), water (2.5%) and TIBS (triisobutylsilane, 2.5%) (v/v/v) with concomitant removal of acid-labile protecting groups. For cleavage of TOC(Dde), this TFA-solution was diluted with dichloromethane in a 1:1 ratio in order to avoid breakdown of the linker unit.

Cyclization of the freshly cleaved peptides was performed in THF containing 5–10% of 5 mM ammonium acetate. The solution was buffered to pH = 7 with saturated $NaHCO_3$ and H_2O_2 (30%, 100 μL per 200 mg of linear peptide) was added subsequently. Cyclization was usually complete within 30 min as shown by HPLC (high-performance liquid chromatography) control.[19,20] The solvents were then evaporated and the peptides were lyophilized. Based on resin-bound Thr/Thr(ol), yields were ≈96% for TATE(Dde) and ≈86% for TOC(Dde).

For DOTA-derivatization, DOTA (1 equiv., typically 0.4–0.8 mmol) was preactivated with NHS (*N*-hydroxysuccinimide, 1.25 equiv.), EDCI (1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide, 1.25 equiv.) and DIPEA (2 equiv.) in water (1 mL/0.3 mmol) for a minimum of 30 min. Then, TOC(Dde) or TATE(Dde) (0.25 equiv.) dissolved in DMF (1 mL per 0.15 mmol of peptide) were slowly added under vigorous stirring. If after 30 min of stirring redissolution of the peptide after the initial precipitation was not complete, more DMF was added in 100 μL portions. As shown in Figure 1, DOTA-coupled-product to precursor ratios (UV absorption at 220 nm) were 93/7 to 99/1 as soon as 30 min of reaction time. After 2 h, HPLC chromatography[19] revealed complete disappearance of the starting peptide both for DOTATOC(Dde) and DOTATATE(Dde). As demonstrated by HPLC and HPLC–MS of the reaction mixtures, no formation of sideproducts occurred for either peptide, i.e. neither doubly substituted DOTA-species nor coupling products resulting from cross-activation of the C-terminal Thr in TATE(Dde).

The solvents were then evaporated to dryness, methanol (up to 5 mL) was added to the residue and the resulting suspension was centrifuged. The peptide dissolved in the supernatant was thereafter precipitated using Et_2O (diethyl ether) and dried in vacuo. For final Dde-deprotection, the DOTA-peptide was dissolved in DMF containing 2% of hydrazine hydrate. After 10 min, the peptide was precipitated using Et_2O and dried

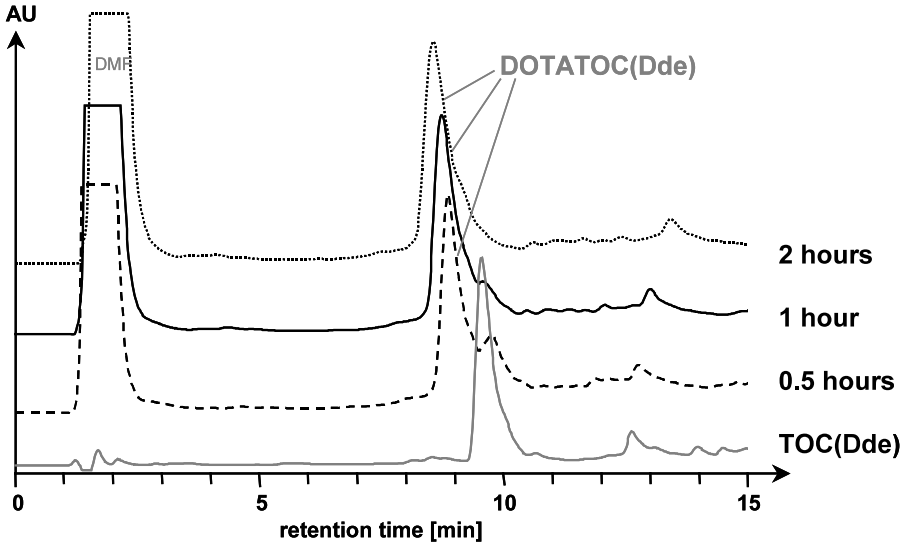

Figure 1. Analytical HPLC reaction control[19] (UV detection at 220 nm) of the formation of DOTATOC(Dde) (DOTA-Lys^5(Dde)-Tyr^3-octreotide) from TOC(Dde) (Lys^5(Dde)-Tyr^3-octreotide) after 0.5, 1 and 2 h of reaction time.

in vacuo. Final product purification was performed using preparative reversed-phase HPLC.[19] DOTATOC and DOTATATE were obtained in purities of 98–99% and in overall yields of 38–52% based on the amount of unpurified protected starting peptide used for the DOTA-coupling reaction. Yields are comparable with those obtained with monoreactive DOTA-analogs and are mainly limited by losses during preparative HPLC.

In conclusion, the present method for the synthesis of DOTATOC and DOTATATE has certain advantages over those previously reported: (1) It utilizes cheap and readily available starting materials, (2) it is rapid (max. 2 h instead of 20–72 h[11,14]), (3) yields of the coupling step are >98%, and (4) no sideproducts have been observed, i.e. the coupling method is compatible with the presence of the unprotected C-terminus in Tyr^3-octreotate.

Therefore, this method represents a synthetically simple and straightforward alternative to the currently used syntheses of DOTA-functionalized peptide analogs and is preferable from an economic point of view.

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0.1% TFA (v/v) in acetonitrile (solvent B) at a constant flow of 1 ml/min. For Lys[5](Dde)-protected peptides, the gradient was 30–60%, for fully deprotected peptides 20–50% B in 15 min. UV-detection was performed at 220 nm using a 206 PHD UV–vis detector. Preparative RP-HPLC was performed on the same HPLC system using either a Multospher 100 RP 18-5 (250×10 mm) column (gradient: 22–45% B in 30 min) at a constant flow of 5 ml/min or a J'Sphere ODS H80 (4 μm, 150×20 mm) column (gradient 18–40% B in 20 min) at a constant flow of 10 ml/min.

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