

Application Note 84:

Efficient Fast-Flow SPPS of Deca-arginine (R10)

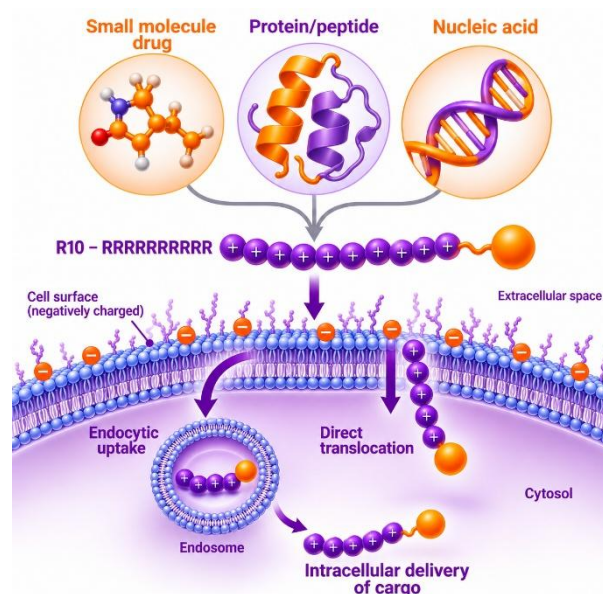
Abstract

Polyarginine peptides present a distinctive challenge for solid-phase peptide synthesis because efficient coupling must be maintained through repeated additions of the same highly basic amino acid, while minimising activation-related side reactions.

In this application note, deca-arginine (R10), comprising ten consecutive arginine residues, was synthesized using Fast-Flow SPPS on the Vapourtec Peptide-Builder™. The synthesis was performed at 0.17 mmol scale using Rink Amide TentaGel S resin and DIC/Oxyma activation.

The complete peptide was synthesized in approximately 3.5 hours. Real-time monitoring showed consistent peptide-chain growth throughout the synthesis, with no evidence of significant resin aggregation. Following cleavage, HPLC analysis gave R10 with a crude purity of 89 %.

The results demonstrate the ability of Fast-Flow SPPS to maintain efficient repetitive coupling across a challenging polyarginine sequence.



For more details, please contact:

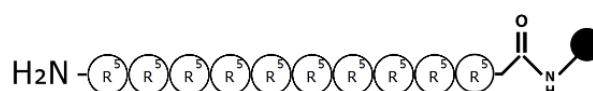
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Background

Deca-arginine (R10) is a short, highly cationic peptide comprising ten consecutive arginine residues:



R10 belongs to the family of arginine-rich cell-penetrating peptides (CPPs), which are of considerable interest as potential delivery vehicles for therapeutic molecules. The guanidinium-rich structure of these peptides promotes interactions with negatively charged components of the cell surface and can facilitate cellular uptake. As a result, arginine-rich CPPs have been widely investigated for the intracellular delivery of molecules that would otherwise cross cell membranes inefficiently, including

small-molecule drugs, peptides, proteins and nucleic acids [1].

This ability has generated interest in CPP-based approaches to drug delivery, including the delivery of macromolecular therapeutics and gene-modulating agents. However, efficient cytosolic delivery, endosomal escape and selective targeting remain important challenges in translating CPP-based delivery systems into therapeutic applications [2].

Alongside its interest as a cell-penetrating peptide, R10 also provides a useful model sequence for evaluating synthetic performance. Although only ten residues long, its repetitive sequence provides a demanding test of solid-phase peptide synthesis because efficient and reproducible coupling must be maintained through ten consecutive arginine additions. Any reduction in coupling efficiency can lead to the progressive accumulation of deletion sequences, particularly R9 and other closely related impurities. Arginine presents an additional challenge during Fmoc SPPS because activated Fmoc-Arg(Pbf)-OH is susceptible to intramolecular cyclisation (lactamisation). This side reaction competes with productive coupling, reducing the amount of activated arginine available for incorporation and potentially increasing deletion impurities. With R10, this challenge is repeated during every successive arginine coupling.

R10 is also highly basic and polar, which can complicate chromatographic analysis of the crude product and the separation of closely related deletion sequences.

R10 therefore provides a useful model for evaluating whether Fast-Flow SPPS can maintain

efficient repetitive arginine coupling while minimising activation-related side reactions and the accumulation of deletion impurities.

Synthesis Conditions

R10 was synthesized at 0.17 mmol scale on the Vapourtec Peptide-Builder™ using Rink Amide TentaGel S resin.

Fmoc-Arg(Pbf)-OH was coupled using DIC/Oxyma activation under the following conditions:

Parameter	Condition
Scale	0.17 mmol
Resin	Rink Amide TentaGel S
Amino acid	Fmoc-Arg(Pbf)-OH
Amino acid equivalents	10 eq.
Activation	DIC/Oxyma
Coupling contact time	1.2 min
Coupling temperature	60 °C

All other synthesis parameters followed the standard Peptide-Builder operating protocol.

The complete ten-residue synthesis was achieved in approximately 3.5 hours.



Real-Time Monitoring of Peptide Synthesis

The Variable Bed Flow Reactor (VBFR™) enables changes in resin-bed volume to be monitored throughout peptide synthesis.

During the R10 synthesis, resin-bed volume increased progressively as the peptide chain was extended. No abrupt or disproportionate increase in resin volume was observed, consistent with the absence of significant resin aggregation during the synthesis.

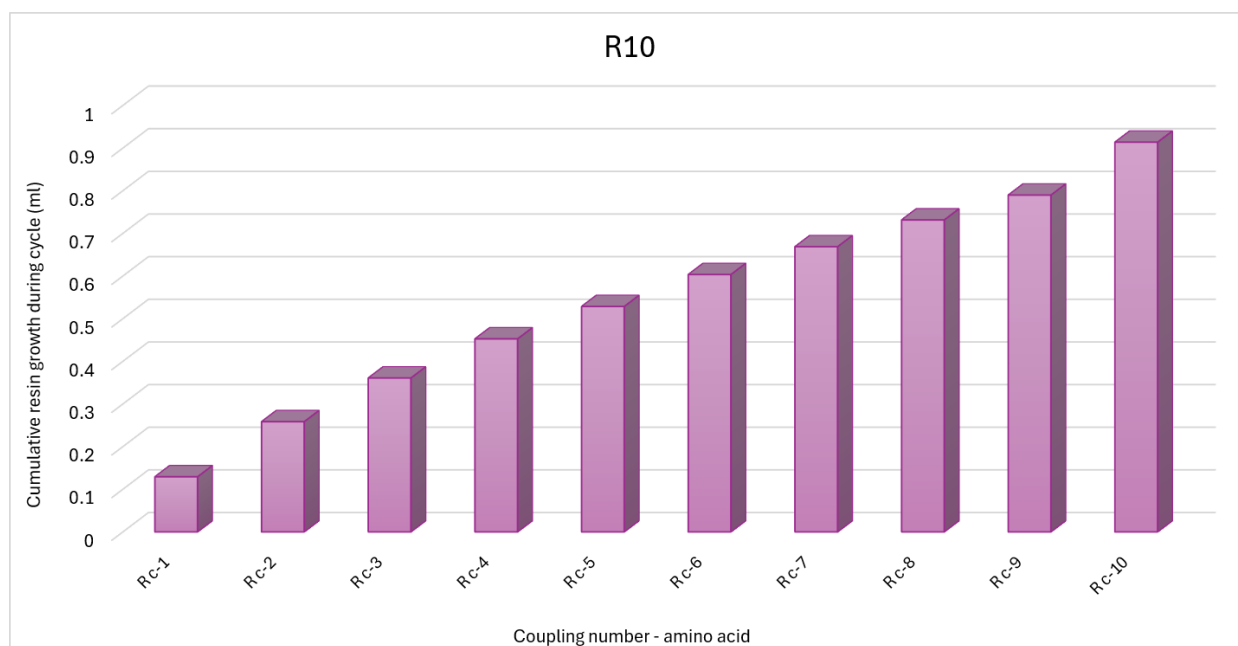


Figure 1: VBFR™ resin-bed volume during synthesis of R10

Real-time Fmoc-UV AUC monitoring provided an additional measure of synthesis progression. The Fmoc response remained consistent across the ten successive arginine additions, supporting continued peptide-chain growth throughout the synthesis.

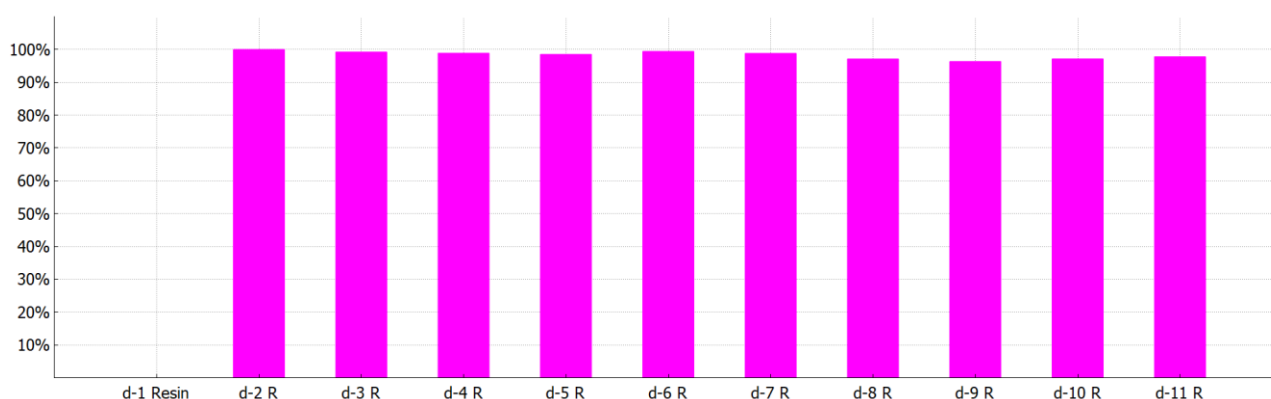


Figure 2: Fmoc-UV response during synthesis of R10

Taken together, the VBFR resin-volume and Fmoc-deprotection data indicate consistent progression of the synthesis through all ten consecutive arginine residues without evidence of significant aggregation.

Analytical Characterisation

Following synthesis, the resin was dried and a representative portion was cleaved. The crude peptide was precipitated in diethyl ether to give a white solid before analysis by HPLC.

Initial reversed-phase HPLC analysis using a conventional water/acetonitrile mobile phase containing 0.1 % TFA did not provide sufficient separation between R10 and the principal R9 deletion impurity.

Improved separation was achieved using heptafluorobutyric acid (HFBA) as an ion-pairing additive. HFBA is a hydrophobic anionic ion-pairing reagent that interacts strongly with positively charged basic residues, and its use in reversed-phase chromatography of oligoarginine peptides has previously been reported [3]. Under these conditions, R10 and R9 were sufficiently resolved to allow their relative peak areas to be determined. The resulting chromatogram gave a crude R10 purity of 89 %.

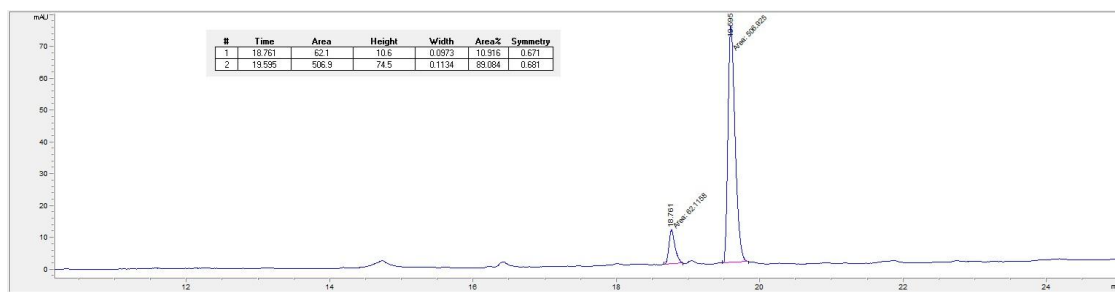


Figure 3: HPLC analysis of crude R10: 89 % purity

The identity of the target peptide was independently confirmed by LC–MS. A formic-acid-based mobile phase was used for compatibility with mass spectrometry. Although this system did not chromatographically resolve R10 completely from R9, mass analysis of the overlapping region confirmed the expected molecular masses corresponding to both peptides.

The two analytical techniques therefore provided complementary information: HFBA HPLC enabled chromatographic separation and determination of the relative peak areas of R10 and the principal R9 impurity, while LC–MS confirmed their molecular identities.

Results and Discussion

Summary of Results:

Result	Outcome
Peptide	H ₂ N-RRRRRRRRRR-CONH ₂
Synthesis scale	0.17 mmol
Synthesis time	~3.5 hours
Crude HPLC purity	89 %
Crude material recovery	52 mg from 280 mg peptidyl resin
Apparent gravimetric recovery	79 % (free-peptide basis)*
Estimated crude material from complete batch	~210 mg
Significant aggregation observed	No
Product identity	Confirmed by LC–MS

The apparent gravimetric recovery is calculated relative to the theoretical mass of R10-NH₂ and does not correct for trifluoroacetate counterions, residual water or solvent in the isolated material. As the peptide was recovered following TFA cleavage, the value should therefore not be interpreted as an absolute molar peptide yield.

Despite requiring ten consecutive arginine couplings, the synthesis maintained consistent peptide-chain growth and produced R10 with 89 % crude purity.

A representative 280 mg portion of the peptidyl resin was cleaved, yielding 52 mg of crude R10 following precipitation. This corresponds to an estimated crude recovery of approximately 210 mg from the complete batch.

Conclusions

Deca-arginine (R10) was successfully synthesized using Fast-Flow SPPS on the Peptide-Builder™ at 0.17 mmol scale. The complete ten-residue synthesis was achieved in approximately 3.5 hours using DIC/Oxyma activation and a coupling contact time of 1.2 minutes.

Real-time VBFR and Fmoc-UV monitoring showed consistent peptide-chain growth throughout the ten consecutive arginine additions, with no significant aggregation signature observed. Following cleavage, HPLC analysis gave R10 with a crude purity of 89 %, with R9 identified as the principal deletion impurity. LC-MS independently confirmed the expected molecular identities of both components.

These results demonstrate that efficient repetitive arginine coupling can be maintained during Fast-Flow SPPS, enabling rapid synthesis of a

challenging polyarginine sequence with high crude purity.

References

1. Anjous, R., Kavyashree, P., Saha, A., Strategies to improve intracellular delivery of arginine-rich cell-penetrating peptides, J Mater Chem B. 2026;Mar 25;14(12):3596-3619.
2. Sun Z, Huang J, Fishelson Z, et al. Cell-Penetrating Peptide-Based Delivery of Macromolecular Drugs: Development, Strategies, and Progress, Biomedicines. 2023;Jul 12;11(7):1971.
3. Xie, W.; Qin, X.; Teraoka, I.; Gross, R. A. Comparison of retention behavior of oligolysine and oligoarginine in ion-pairing chromatography using heptafluorobutyric acid. Anal. Bioanal. Chem. 2013, 405, 9739–9746. DOI: 10.1007/s00216-013-7397-9.