Silicon-containing amino acids for ^{18}F labeling of neurotensin analogues targeting NTS$_1$ receptor

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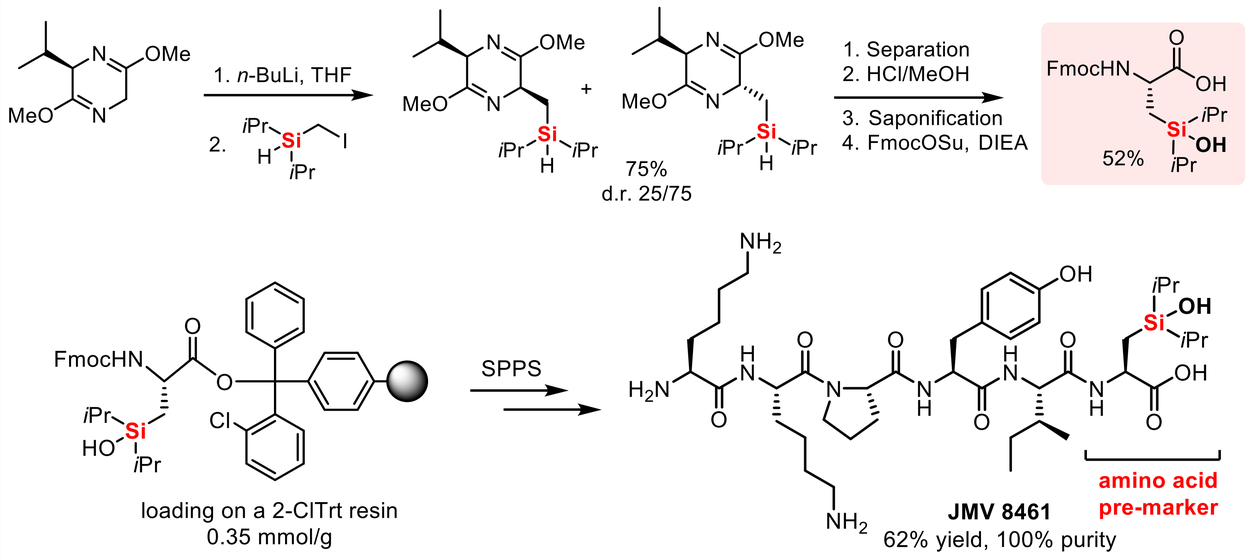
INTRODUCTION

Targeting the neurotensin receptor-1 (NTS$_1$) is of great interest for the treatment of several cancers with aggressive phenotypes, including pancreatic adenocarcinoma, triple-negative breast cancer and colorectal cancer.[1] In this field, we have reported ^{68}Ga-radiolabeled NT[8-13] analogue conjugates, stabilized by the non-natural silylated amino acid (L)-trimethylsilylalanine (TMSAla) and sequence modifications, for PET imaging of tumors over-expressing NTS$_1$[2] or NTS$_2$[3] receptors. Herein, we present new NT[8-13] analogues designed for ^{18}F labeling, with a half-life of 110 min enabling later imaging than ^{68}Ga (half-life = 68 min) to potentially visualize more lesions with a better signal-to-noise ratio.

Silicon-containing amino acid pre-marker

Alkylation of the bis-lactim ether with diisopropylsilane led to diastereoisomers (*cis*/*trans* 25 :75) in 75% yield. After separation, hydrolysis of the *trans* diastereoisomer, saponification and protection, the N-Fmoc protected amino acid was isolated in 52% yield (Scheme 1). SPPS was then performed on a 2-chlorotrityl resin pre-loaded with this silanol amino acid pre-marker, which is adapted for radiolabeling by the formation of an Si-^{18}F bond.



Scheme 1. Diastereoselective synthesis of silanol amino acid and SPPS

NT[8-13] analogue conjugates with an arylsilane

We have also developed conjugates by coupling di-*t*-butyl or diisopropyl arylsilane to NT[8-13] analogues containing TMSAla in the C-terminal position, via an APAc or (β-Ala)$_2$ linker in yield of 10-59% and purity of over 96% (Figure 1). To increase resistance to enzymatic degradation, analogues containing a reduced bond between the two lysines [Lys^{8}CH$_2$NHLys^9] were also synthesized.

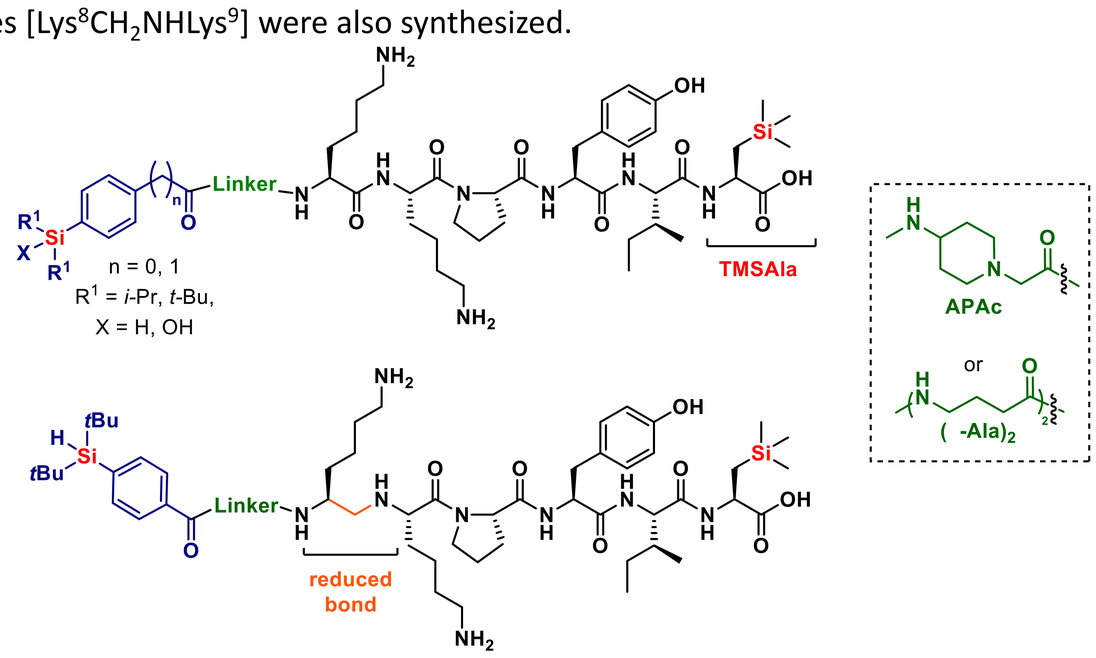
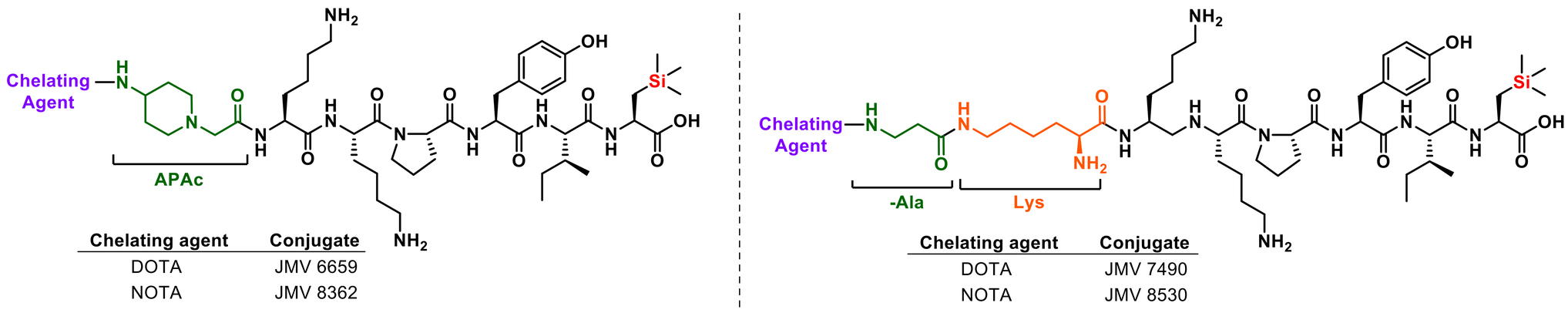


Figure 1. Structure of conjugates with an arylsilane attached at the N-terminal position

NT[8-13] analogues designed for ^{18}F-radiolabeling with a macrocycle

Conjugates JMV 8362 and JMV 8530 were radiolabeled with ^{18}F by the aluminium fluoride method, as well as with ^{68}Ga and ^{64}Cu for direct comparison. ^{64}Cu is another PET radionuclide with longer half-life (12.7h, available from ARRONAX, Nantes, France). *In vitro* characterization (internalization assay) of **^{68}Ga/^{18}F/^{64}Cu-JMV 8362** and **^{68}Ga/^{18}F/^{64}Cu-JMV 8530** was carried out on HT-29 human colorectal cancer cells over-expressing NTS$_1$. The DOTA reference compounds ^{68}Ga-JMV 6659 and ^{68}Ga-JMV 7490 were used as internal comparators (Figure 2, left). ^{68}Ga-JMV 8530 showed better NTS$_1$-internalization than the DOTA reference compound ^{68}Ga-JMV 7490. ^{68}Ga-JMV 8362 showed similar NTS$_1$-internalization to the reference compound ^{68}Ga-JMV 6659. Both ^{18}F-labeled conjugates showed very high internalization overpassing ^{68}Ga-JMV 6659 and ^{68}Ga-JMV7490. Surprisingly, ^{64}Cu-JMV 8530 showed weaker internalization than ^{64}Cu-JMV 8362 which exhibited long internalization in HT-29 cells (still 25% internalization 24h after incubation).



Chelating agent	Conjugate
DOTA	JMV 6659
NOTA	JMV 8362

Chelating agent	Conjugate
DOTA	JMV 7490
NOTA	JMV 8530

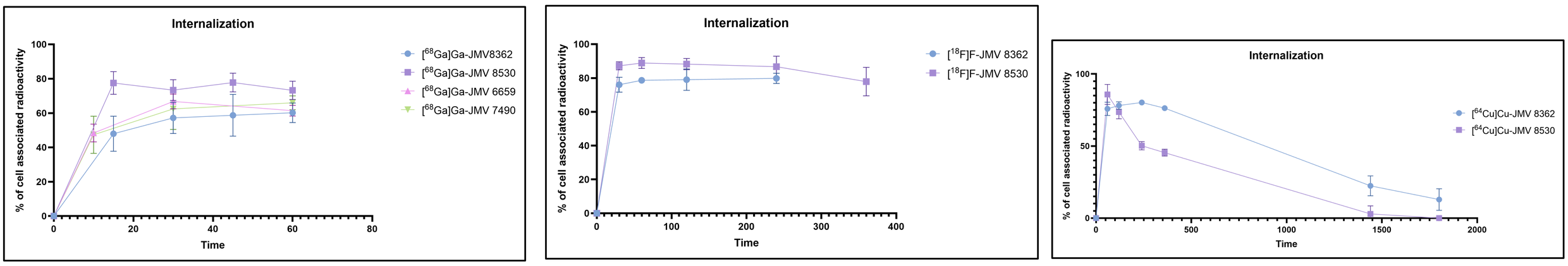


Figure 2. Structure of conjugates and internalization assay of **^{68}Ga/^{18}F/^{64}Cu-JMV 8362** and **^{68}Ga/^{18}F/^{64}Cu-JMV 8530** performed on HT-cells. *Left*: internalization of ^{68}Ga-NOTA-conjugates compared to ^{68}Ga-DOTA-conjugates. *Middle*: internalization of ^{18}F-NOTA-conjugates. *Right:* internalization of ^{64}Cu-NOTA-conjugates

CONCLUSION

We explored an innovative strategy for ^{18}F-radiolabeling of NT[8-13] analogues by introducing of a new class of silylated amino acid as pre-marker for fluorination, through the formation of a Si-^{18}F bond which should be further investigated for direct late labeling, either internally (C-terminal position) or prosthetically at the N-terminal position. As preliminary results to compare radionuclides, we have successfully developed new conjugates by coupling NT[8-13] analogues to chelating agents for radiolabeling with ^{68}Ga/^{18}F/^{64}Cu. These results show the the superiority of the NOTA over the DOTA and underline the value of developing fluorine labeling.

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