How to cite: *Angew. Chem. Int. Ed.* **2022,** *61,* e202205368
International Edition: doi.org/10.1002/anie.202205368
German Edition: doi.org/10.1002/ange.202205368

Peptide Modification

Selective Stepwise Arylation of Unprotected Peptides by Pt^{IV} Complexes

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Abstract: $LPt^{IV}F(Aryl)$ complexes bearing a bulky bidentate 2-[bis(adamant-1-yl)phosphino]phenoxide ligand (L) demonstrate excellent reactivity and selectivity in the arylation of X–H (X=S, N) bonds of amino acid residues in unprotected peptides under mild, including aqueous, conditions. Stepwise addition of these complexes allowed a convenient one-pot introduction of different aromatic groups in the X–H bonds of Cys and N terminus. Pt^{IV} reagents can also be used to further arylate N–H bonds in Lys and Trp providing access to peptides bearing multiple aromatic groups.

Synthetic bioconjugation of peptides and proteins is a rapidly growing research area with a variety of applications in biology and medicine.[1] In recent years, transition metal-mediated bioconjugation has received considerable attention thanks to the developed understanding of the reactivity of transition metal complexes, particularly with regard to catalytic transformations.[2,3] Unsurprisingly, the majority of the reactions and transition metal reagents used in the bioconjugation resemble those most commonly employed in catalysis.[4] Generally, these reactions utilize the redox chemistry, carbene insertions or cross-coupling of modified amino acid residues assisted/catalyzed by complexes of Cu, Au, Pd, Ni, Rh or Ru.[5]

Coupling reactions of unmodified amino acid residues is more attractive and typically involve ubiquitous X–H bonds (X=S, N or O), although other functionalization reactions are also known.[6–8] The S–H bond in the Cys side chain has been a particularly popular coupling target.[9] For example, Pentelute and Buchwald and co-workers reported Pd^{II} aryl complexes bearing bulky phosphine ligands as coupling partners for the Cys S–H bonds in peptides and proteins (Figure 1a).[10] However, finding organometallic reagents that can differentiate between the less reactive but much more abundant N–H bonds represents a significant challenge. While Pd^{II} aryl complexes were employed in the arylation of the ε-NH_2 group of Lys residues in peptides under basic conditions (Figure 1a),[11] competitive coupling involving NH_2 groups in Asn, Arg and N terminus was also observed. Very recently, Ball et al. reported Cu-mediated selective arylation of a terminal NH_2 group, however, the reaction was limited to o-substituted electron-poor aromatic rings.[12] Thus, the design of new metal complexes capable of differentiating between the various types of N–H bonds in unprotected peptides under mild conditions remains an important area of research. Interestingly, despite their rich and well-established C–X reductive elimination chemistry,[13] group 10 M^{IV} metal complexes have not been used in the bioconjugation reactions. In particularly, Pt^{IV} complexes showed high selectivity in the formation of C–X bonds under mild conditions,[14,15] however no applications of such reactivity in biologically relevant systems have been reported.[16] Very recently, we have shown that sterically demanding phosphino-phenoxide $(P–O)Pt^{IV}$ complexes **1** bearing primary amine ligands undergo mono- and di-arylation reactions in very high yields at room temperature or mild heating ($\leq 40\,^{\circ}C$, Figure 1b).[17] Mild arylation of S–H and O–H bonds was also observed with related Pt^{IV} complexes.

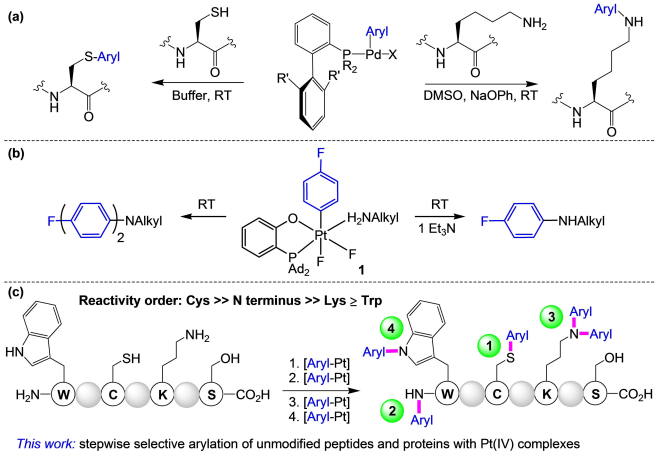

Figure 1. a) Pd^{II}-assisted arylation of Cys and Lys residues. b) Mild primary amine arylation by Pt^{IV} complexes. c) This work: stepwise selective arylation of X–H bonds in peptides with Pt^{IV} complexes.

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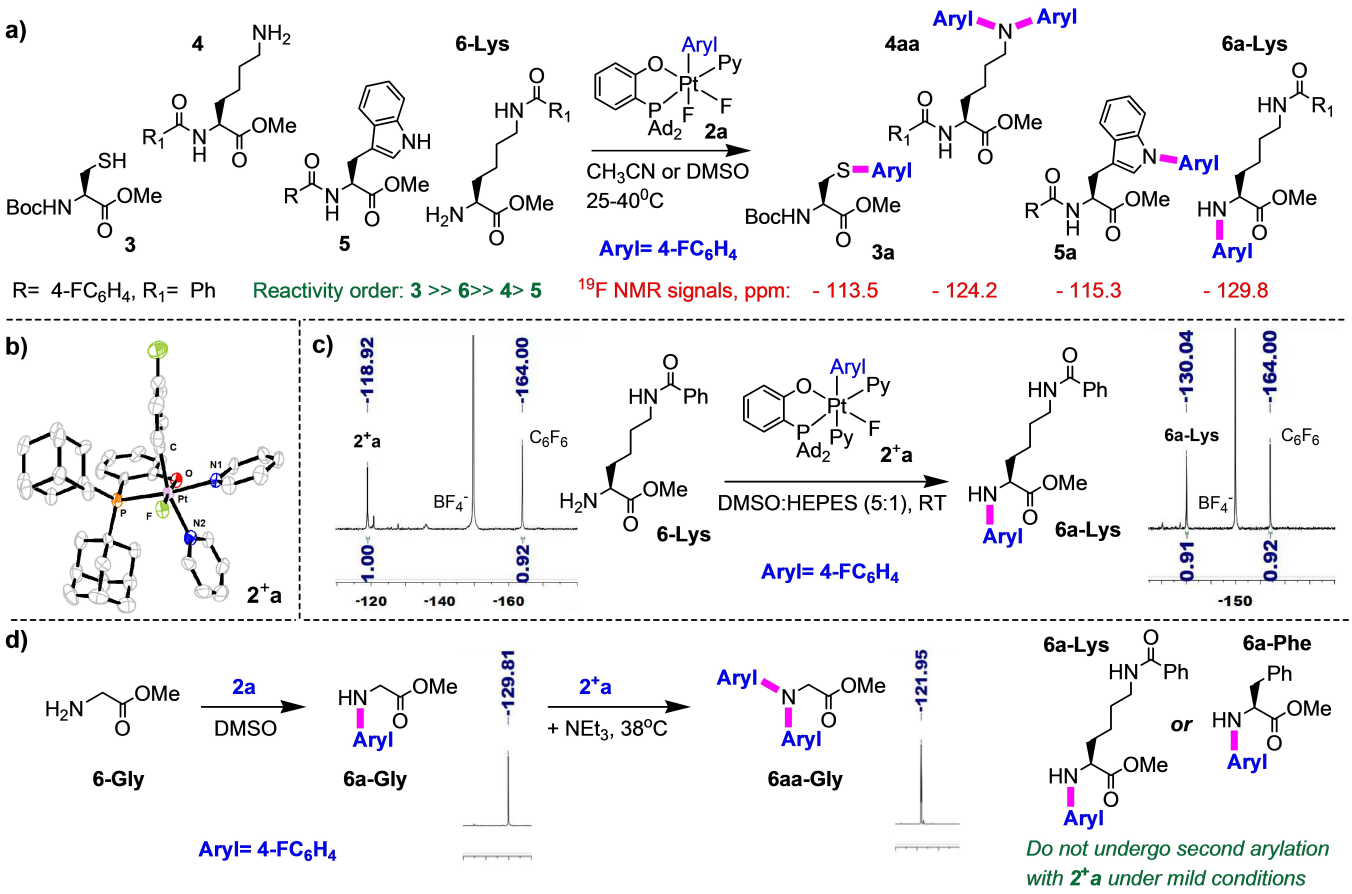
These findings prompted us to explore relative reactivity of different X–H functional groups and potential applications of Pt^{IV} complexes in bioconjugation chemistry. Here, we present the Pt^{IV}-mediated selective arylation of functional groups in unprotected peptides, including an unprecedented stepwise arylation by the same type of metal complexes (Figure 1c).

Initially, we explored the reactivity of amino acid derivatives in the aryl-X coupling reactions with complex **2a**,[17] the pyridine analog of **1**. We found that only four X–H groups undergo arylation reaction under mild conditions (CH_3CN or DMSO, 25–40 °C, Scheme 1a) in the following reactivity order: S–H (Cys, **3**) ≫ α-N–H (**6-Lys**) ≫ ε-N–H (Lys, **4**) > N–H (Trp, **5**).[18] For example, in DMSO, the S–H arylation is completed within several minutes, while monoarylation of an α-NH_2 group typically takes place within an hour. Complete arylation of an ε-NH_2 group in Lys requires about 6 hrs, with both N–H bonds being replaced. The strong preference for the α-NH_2 group in N terminus vs. the ε-NH_2 group in the Lys side chain contrasts that of the Pd^{II} complexes[11] and can be attributed to higher acidity of the α-NH_2 group coordinated to an electrophilic Pt^{IV} center, which facilitates the deprotonation step prior to the C–N reductive elimination.[17] A similar preference for the less basic aniline over ε-NH_2 group in Lys was very recently reported for the Pd^{II}-assisted arylation of the aniline-modified peptides, with the coordinated aniline showing higher reactivity.[19] The indole N–H bond of a Trp moiety is the least reactive among the four functional groups, the reaction being completed after ca. 8 h at 38 °C.

The N–H bond in proline reacted very slowly, suggesting that proline-terminated peptides can be also arylated. The OH groups (Ser, Tyr) required heating to 60–65 °C to undergo the arylation reaction, while the side NH_2 groups in amides (Asn) and guanidine (Arg) were unreactive. Overall, the established reactivities of amino acid derivatives predict that high selectivity for the Cys and N terminus can be achieved in the peptide arylation, whereas the Lys and Trp residues can also be arylated albeit less selectively (see below). Importantly, very high sensitivity of the 4-FC_6H_4 signal in the ^{19}F NMR spectrum to the nature of the substituent in the *para*-position proved invaluable in determining the selectivity of the arylation reactions.

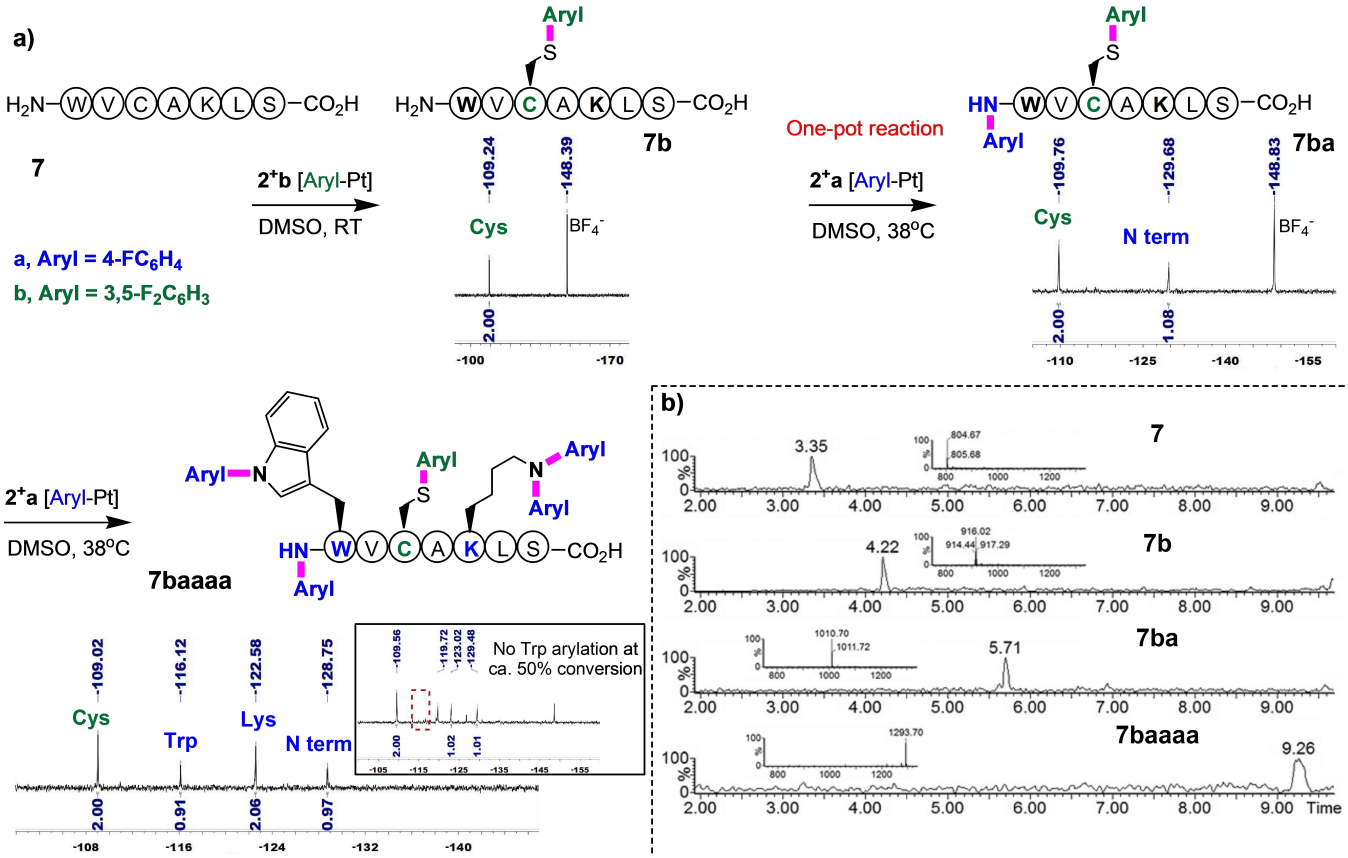
Although **2a** showed good reactivity, it has a limited thermal stability and slowly decomposes in protic solvents at room temperature. To solve these issues, we prepared and crystallographically characterized the cationic complex **2⁺a**, where the reactive F ligand trans to the aryl group is



Scheme 1. a) Examples of amino acid derivatives undergoing facile arylation with a Pt^{IV}-Aryl complex. b) ORTEP view of a cation of **2⁺a**. c) Representative arylation of an α-NH_2 group by complex **2⁺a** under aqueous conditions showing ^{19}F NMR spectra of the crude mixture. d) Stepwise double arylation of the Gly α-NH_2 group by **2⁺a**.

replaced by a neutral labile pyridine ligand (Scheme 1b).[20] Several cationic Aryl-Pt^{IV} complexes were also prepared to explore the selective stepwise peptide modification with different aromatic groups. While somewhat less reactive than the parent **2**, the cationic complexes **2**$^+$ can be stored and handled in air. They also showed compatibility with the aqueous media, including complex buffer mixtures. For example, facile α-N–H arylation of **6-Lys** with **2**$^+$**a** was observed in a DMSO:HEPES 0.2 M buffer mixture[21] within 2 hrs at RT (Scheme 1c). Finally, we found that the smallest amino acid Gly is the only amino acid that can undergo a stepwise double arylation reaction at the α-NH_2 group under relevant conditions (**6aa-Gly**, Scheme 1d),[22] thus potentially allowing decoration of the Gly-terminated peptides with two aromatic groups.

With these results in hand, we moved to explore selective X–H arylation of unmodified peptides. Synthetic peptide **7** with the randomly placed Cys, Lys and Trp residues was prepared and reacted with the Pt^{IV} complexes **2**$^+$ (Scheme 2). The reaction progress was monitored by the ^{19}F NMR spectroscopy and LCMS. As expected, the modifications of the Cys S–H bond and N terminus proceeded readily with the stepwise introduction of aryls 3,5-$F_2C_6H_3$ (Cys, complex **2**$^+$**b**) and 4-FC_6H_4 (N terminus, complex **2**$^+$**a**) in one pot and no evidence for the arylation of other X–H bonds in **7** was observed by the ^{19}F NMR spectroscopy (Scheme 2).[23] The ^{1}H NMR spectrum of purified **7ba** showed the presence of two new aromatic groups (Figure S74). While both reactions can proceed at RT, raising the temperature to 38 °C for the N terminus arylation step shortens the reaction time to 2 hrs and the Pt^{II} byproduct conveniently precipitates from the solution under these conditions.[24] Although highly selective for the N terminus position, Pt^{IV} reagents can be further used to arylate the remaining N–H bonds of amino acid residues in **7ba**. Monitoring the reaction between **2a** and isolated **7ba** revealed the ^{19}F NMR spectrum consistent with the initial formation of **7baaa**, the product of the selective double arylation of the Lys residue. Continuing the reaction over 50 % conversion led to the appearance of a signal at −116.1 ppm due to the competing Trp NH arylation.[25] Considering that Trp is the least abundant amino acid among the naturally occurring common amino acids, it should be possible to use Pt^{IV} complexes also for the modification of a Lys residue in natural peptides and proteins (see below). On the other hand, arylation of the NH bond in a tryptophan residue by Pt^{IV} complexes under mild conditions provides an unprecedented tool for the modification of unprotected peptides.[26] Indeed, using an excess of **2**$^+$**a** (overall 10 equivalents for the last two steps), it was possible to complete the arylation sequence, as evidenced by the disappearance of the indolic N–H proton



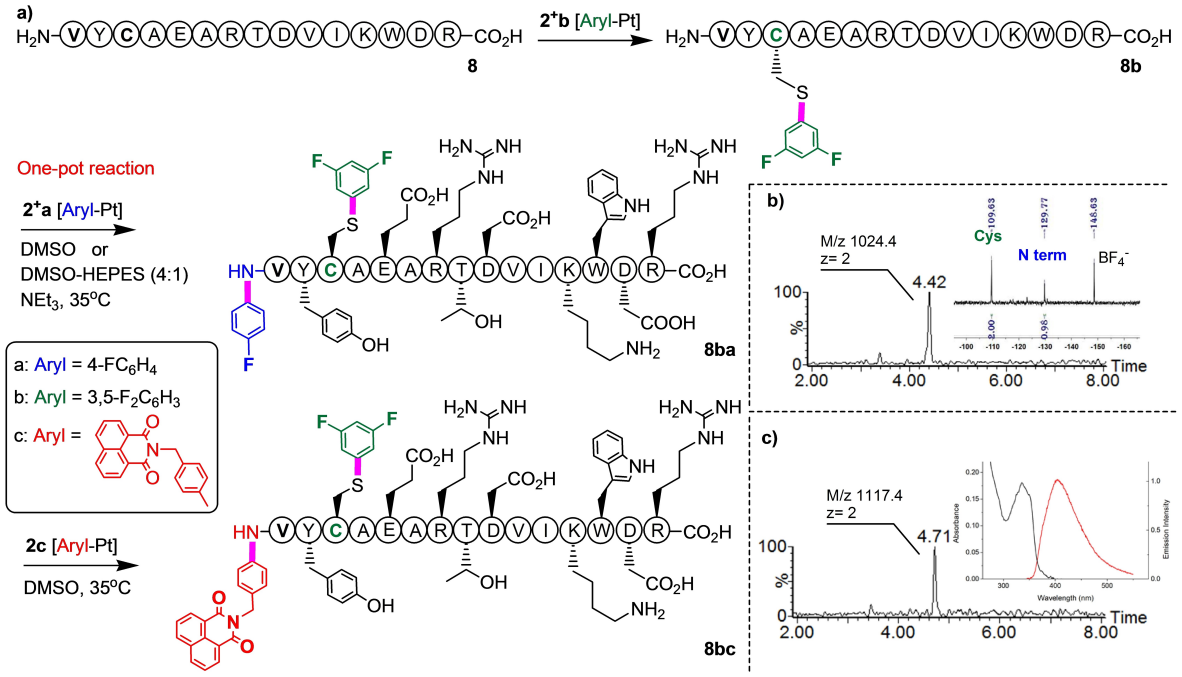
Scheme 2. a) Stepwise selective arylation of peptide **7** (8.3 mM in DMSO) monitored by the ^{19}F NMR spectroscopy (inset shows crude mixture of Lys arylation step at ca 50% conversion). b) LCMS analysis of the reaction mixture.

in the ^{1}H NMR spectrum (Figure S77), and obtain the fully arylated peptide **7baaaa** (Scheme 2).

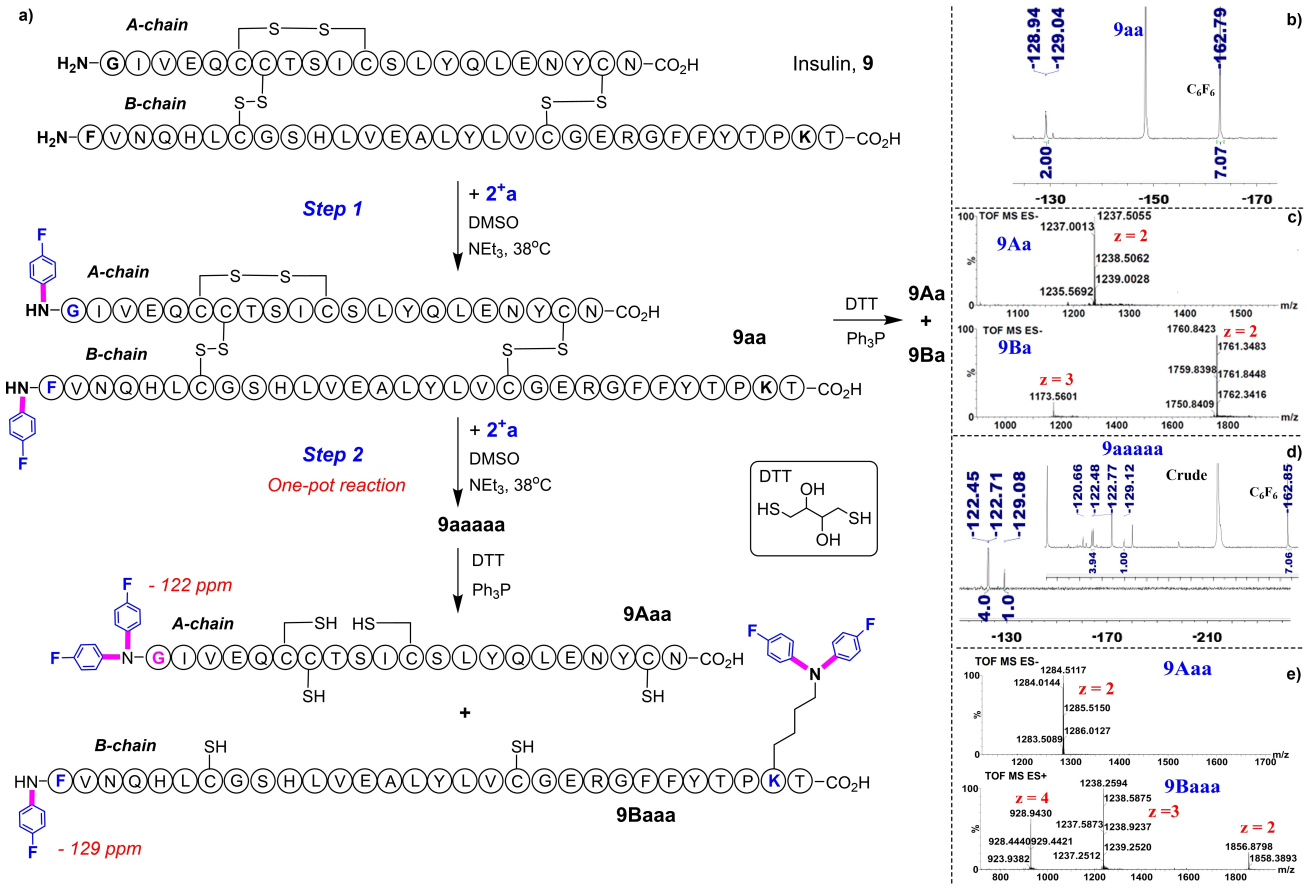
To evaluate the compatibility of the Pt^{IV}-mediated arylation with more complex peptide substrates, particularly under aqueous conditions, we studied the reactivity of complexes $\mathbf{2}^{+}$ with the synthetic peptide **8**, containing 15 amino acids in a 4:1 mixture DMSO-HEPES buffer (0.1 M) (Scheme 3a).[21,27]

Although both, $\mathbf{2}^{+}$ and **8** showed limited solubility in this mixture, selective arylation of the Cys residue and N terminus proceeded readily via a stepwise addition of $\mathbf{2}^{+}$**b** and $\mathbf{2}^{+}$**a** to give **8ba** in a 89 % yield (Scheme 3a, b). Further functionalization of the remaining amine functions was sluggish under these conditions, presumably due to the heterogeneity of the reaction mixture. Nevertheless, these results demonstrate that the selective Pt^{IV} arylation of peptides is compatible with the aqueous media, a general requirement for potential applications in protein bioconjugation. Because the observed selectivity results from the inherent reactivity preferences of the Pt^{IV} complexes, identical or similar aromatic groups can be used for the different types of X–H bonds.[28] Furthermore, while the ^{19}F NMR tag is helpful in determining relative reactivity and selectivity of stepwise arylation, the reactions are clearly not limited to simple fluoroaromatics. For example, using **2c** in the second arylation step it was possible to introduce a fluorescent naphthalimide group at the N terminus of the peptide, giving 87 % of the diarylated peptide **8bc** which was isolated and characterized by the ^{1}H and ^{19}F spectroscopy, LCMS and UV-fluorescence spectroscopy (Scheme 3a, c and Supporting Information). To confirm the selective arylation of the N terminus, compound **8bc** was treated with an aminopeptidase enzyme in HEPES buffer.[12] No changes in **8bc** was observed even after 3 days, while the parent peptide **8** reacted after 15 minutes under the same conditions.[18]

Finally, we applied the Pt^{IV} complexes in the arylation of a large natural peptide. As a target, we chose human insulin **9**, a 51-meric protein containing two peptide chains, each with an unprotected N terminus. With six Cys residues engaged in disulfide linkages, we assigned these N termini as the most reactive sites. Because the A chain in **9** has Gly at its N terminus, we also envisaged potential double arylation at this position under more forcing conditions. In addition, insulin contains a single Lys residue, which should also be reactive in the arylation by Pt^{IV} complexes. Gratifyingly, monitoring the reaction between **9** and 2.5 equiv of $\mathbf{2}^{+}$**a** in DMSO for 3 hrs at 38 °C by the ^{19}F NMR spectroscopy showed the conversion of insulin to the product **9aa** bearing a 4-FC_6H_4 group at each of the N termini (Scheme 4a). The product shows two signals appearing slightly apart at ca. −129 ppm (Scheme 4b), further highlighting the sensitivity of the ^{19}F NMR spectroscopy in determining the selectivity of the N–H arylation. No signals due to the formation of the 4-FC_6H_4-S bonds at ca. −112 ppm was observed testifying to stability of the disulfide bridges under the reaction conditions. Addition of DTT (dithiothreitol) to a solution of **9aa** led to the reduction of the S–S bonds and formation of two separate chains (**9Aa** and **9Ba**), each containing one 4-FC_6H_4 group (Scheme 4c). Interestingly, further addition of an excess (7–8 equiv) of $\mathbf{2}^{+}$**a** and NEt_3 to **9aa** led to a gradual decrease of one of the signals at ca. −129 ppm with concomitant appearance of a signal at ca. −122 ppm until the 1:2 ratio between the two signals was established. These observations indicate sequential double arylation of the Gly



Scheme 3. Stepwise selective arylation of peptide **8** (2 mM in DMSO or DMSO-HEPES): a) Synthesis of peptides **8b**, **8ba** and **8bc**. b) LCMS and ^{19}F NMR (inset) spectra of **8ba**. c) LCMS and UV-Fluorescence (inset) spectra of **8bc**.



Scheme 4. Stepwise selective arylation of human insulin **9** (2 mM in DMSO): a) Synthesis of **9aa** bearing two 4-FC_6H_4 groups and **9aaaaa** bearing five 4-FC_6H_4 groups, and their DTT-assisted conversion to two separate chains (A and B). b) ^{19}F NMR spectrum of crude **9aa**. c) MS spectra of chains **9Aa** and **9Ba** obtained after the reaction between **9aa** and DTT (see text). d) ^{19}F NMR spectrum of **9aaaaa** (inset-spectrum of the crude mixture). e) MS spectra of chains **9Aaa** and **9Baaa** obtained after the reaction between **9aaaaa** and DTT.

N terminus of the A chain (ca. −122 ppm, cf. Scheme 1d) while the Phe N terminus remaining with a single 4-FC_6H_4 group (ca. −129 ppm). In addition, another signal simultaneously appeared at ca. −122 ppm (2F) indicating double arylation of the B29 Lys residue (Scheme 4d). The final ^{19}F spectrum showed three singlets in a 2:2:1 ratio suggesting overall five aromatic groups attached to the insulin molecule **9aaaaa**. Integration vs the internal C_6F_6 confirmed the quantitative yield of the arylation reactions. Cleavage of the S–S bonds with excess of DTT gave the separate peptide chains, **9Aaa** and **9Baaa**, bearing two and three 4-FC_6H_4 groups, respectively (Scheme 4e). These results demonstrate that Pt^{IV} reagents can be used in selective stepwise arylation of N termini and Lys residues in a complex natural polypeptide molecule, such as insulin. The disulfide bridges remain stable throughout the reaction, although the current work-up protocol is incompatible with isolation of peptides containing this moiety.

Overall, our studies demonstrate the potential for the utilization of well-defined Pt^{IV} complexes in chemoselective arylation of amino acid residues and N termini of unprotected peptides. The determined reactivity trend of Cys ≫ N terminus ≫ Lys ≥ N–H Trp allows for the introduction of different aromatic groups at the selected sites of these biologically relevant molecules. The reactions take place under mild conditions and are compatible with aqueous solutions. We are currently exploring the applications of Pt^{IV} complexes in peptide bioconjugation, particularly their extension to protein bioconjugation in water.

Acknowledgements

We are grateful to Dr. Noam Tal for help with the mass spectrometry measurements and Dr. Sophia Lipstman for help with the X-ray crystallography. We thank the US-Israel Binational Science Foundation (BSF, grant 2014254), Jerusalem, Israel and Joint NSFC-ISF (grant 2572/17) for supporting this work.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Arylation Reactions · Bioconjugation · Peptides · Platinum Complexes

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Manuscript received: April 12, 2022
Accepted manuscript online: May 10, 2022
Version of record online: June 2, 2022