

Amino Acid–Viologen Hybrids: Synthesis, Cucurbituril Host–Guest Chemistry, and Implementation on the Production of Peptides

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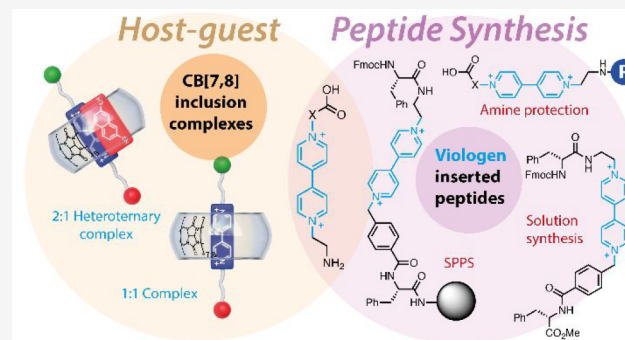


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Supporting Information

ABSTRACT: We present herein the development of a series of viologen–amino acid hybrids, obtained in good yields either by successive alkylations of 4,4′-bipyridine, or by Zincke reactions followed by a second alkylation step. The potential of the obtained amino acids has been exemplified, either as typical guests of the cucurbituril family of hosts (particularly CB[7]/[8]) or as suitable building blocks for the solution/solid-phase synthesis of two model tripeptides with the viologen core inserted within their sequences.



Viologens (Vs), compounds resulting from the diquaternization of 4,4′-bipyridine, are one of the most studied classes of stimuli-responsive moieties in chemistry, mainly due to their synthetic accessibility and adjustable properties, such as reversible redox behavior and π -acceptor character.¹ Consequently, this broad family of organic salts has been extensively used in a variety of practical applications,² including the development of V-based electrochromic materials³ or, more recently, aqueous redox flow batteries.^{4,5}

Furthermore, their high tunability and stimuli-responsiveness have made Vs prime components on the evolution of supramolecular chemistry. For instance, Vs can be found as the key parts of the family of macrocyclic receptors known as ExBoxes developed by Stoddart et al.⁶ or as archetypical guests of relevant hosts⁷ such as aryl-containing coronands,⁸ pillarenes,⁹ or cucurbiturils^{10,11} (referred in this paper as CB[*n*]s, *n* = 7, 8; Figure 1). In particular, the host–guest chemistry of viologens and CB[8] is currently highly significant,¹² as heteroternary complexes can be prepared in a predictable fashion, typically by using a V as first guest and an

electron donor as second (D). Furthermore, the very accessible and reversible first reduction potential of Vs enables the controlled assembly/disassembly of the obtained CB[8]:V:D [3]pseudorotaxane in very convenient experimental conditions. Hence, this redox-controlled “handcuff strategy” has become the weapon of choice not only for the transient heterologation of discrete small molecules by CB[8]^{10–13} but also for the creation of more complex assemblies.^{10–14}

Following our interest in the supramolecular chemistry of pyridinium salts,¹⁵ we initiated a research program focused on the CB[*n*]-based modification of peptides owning appropriate viologen moieties as binding motifs.¹⁶ In this context, we found the lack of a general methodology for the insertion of the V core within peptide sequences, with most of the procedures reported so far being focused on the end-capping or lateral chain modification of the oligomer with the electroactive motif.^{16–18} Consequently, we envisaged the synthesis of a series of viologen–amino acid hybrids (Figure 1) that would potentially allow for their implementation in liquid or solid phase peptide synthesis (L/SPPS) and could retain the characteristics required for the CB[7,8]-based molecular recognition.

Considering the well-established methodology for the synthesis of unsymmetrical Vs,^{2,19} we first tackled the synthesis of a V–amino acid hybrid by direct introduction of the

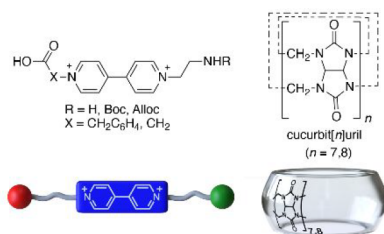


Figure 1. Schematic depiction of the V–amino acid hybrids discussed in this work and of the CB[*n*] (*n* = 7, 8) hosts.

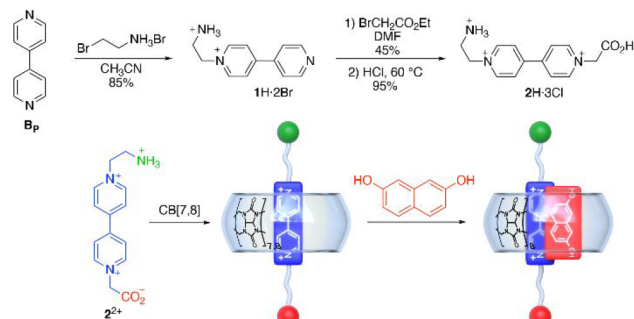
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envisioned functional groups through successive alkylation reactions of the 4,4'-bipyridine (B_p) core. As shown in Scheme 1, the unprotected amino acid 2H·3Cl could be prepared by

Scheme 1. Synthesis of Amino Acid 2H·3Cl and Representation of the Binary $CB[7,8]:2^{2+}$ and Heteroternary $CB[8]:2^{2+}:2,7\text{-DHN}$ Complexes



reaction of B_p with commercially available 2-bromoethan-1-amonium bromide, followed by a second alkylation of the intermediate 1H·2Br with ethyl 2-bromoacetate, and a final step for the hydrolysis of the corresponding ester with aqueous HCl, which produces as well the complete metathesis of the bromide counterions, leading to the trichloride salt in a decent 36% overall yield.²⁰

To test the ability of the obtained amino acid as appropriate guest for $CB[n]$ s, we proceeded to study the complexation of the zwitterionic form 2^{2+} by $CB[7]/[8]$ in buffered aqueous media at $pD = 7$; a fact that would simplify the assessing of the binding interactions by means of 1H NMR spectroscopy.²¹ Both in the case of $CB[7]$ and $CB[8]$, the changes observed in the NMR spectra of guest 2^{2+} upon addition of increasing quantities of host²² are in agreement with the complexation of the V moiety following a fast, but near coalescence, exchange rate on the NMR time scale. In both cases, the complexation-induced shifts (CISs) for equimolar mixtures of host and guest (see, for instance, Figure 2a)²² are fully consistent with the inclusion process producing 1:1 symmetric pseudorotaxanes. In essence, the shielding of the signals attributable to the viologen core of 2^{2+} as well as the slight deshielding of the methylene pendant groups of the guest suggest the expected binding mode with the V core inserted within the cavity of the hosts as in similar systems.¹⁰ ESI-MS spectrometry also verified the formation of the binary complexes, with intense peaks corresponding to the expected species being detected for $[CB[7]:2]^{+2}$ at (m/z) calcd 711.7428, found 711.7434 and for $[CB[8]:2]^{+2}$ at (m/z) calcd 794.7674, found 794.7679.²² Furthermore, UV-vis titration experiments allowed for the assessment of the association constants, with the obtained data fitting appropriately to 1:1 isotherms with K_a ($CB[7]:2^{2+}$) = $(5.7 \pm 0.5)10^5 M^{-1}$ and K_a ($CB[8]:2$) = $(5.2 \pm 0.3) 10^5 M^{-1}$ (Figure 2b), values in good agreement with those previously reported for similar systems.¹⁰

Finally, the obtention of the heteroternary complex between $CB[8]$, 2^{2+} , and 2,7-DHN, as a typical second guest, was also tested by NMR (Figure 2a). Although some of the resonances for the two guests disappear because of a near-coalescence exchange regime on the technique at rt ,²² the problem was surpassed by recording the experiment at 343.15 K for a 1:1:1 mixture of the compounds at 1 mM (Figure 2a), clearly showing the expected CISs for this type of complexes.

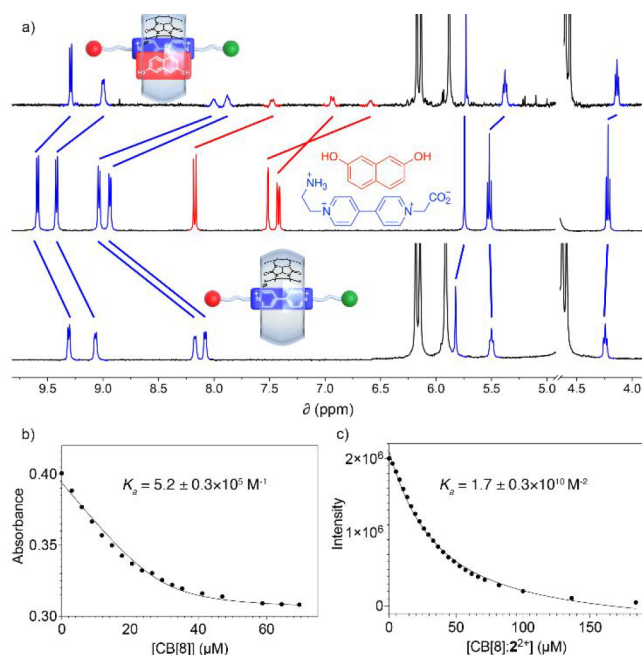


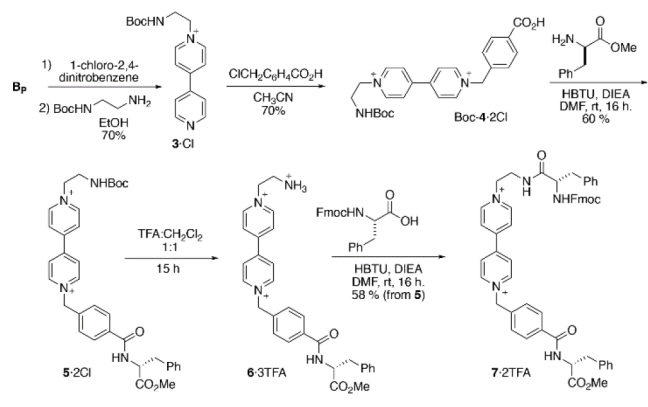
Figure 2. (a) Partial 1H NMR spectra (D_2O , 500 MHz) for equimolar (1 mM) mixtures at 343.15 K of (middle) 2^{2+} and 2,7-DHN, (top) 2^{2+} , 2,7-DHN and $CB[8]$, and (bottom) 2^{2+} and $CB[8]$. (b) Fitting of the observed variation in the absorption at $\lambda = 272$ nm of a $29.4 \mu M$ 2^{2+} solution in phosphate buffer (50 mM, pH = 7.0), upon addition of increasing concentrations of $CB[8]$. (c) Fitting of the observed variation in the fluorescence emission at $\lambda_{em} = 345$ nm of a $10 \mu M$ 2,7-DHN solution in phosphate buffer (50 mM, pH = 7.0), upon addition of increasing concentrations of $CB[8]:2^{2+}$.

Furthermore, the inclusion of 2,7-DHN as a second guest was also corroborated by fluorescence titrations, which allowed us to estimate the overall K_a ($CB[8]:2^{2+}:2,7\text{-DHN}$) = $(1.7 \pm 0.3) 10^{10} M^{-2}$ (Figure 2c).²²

Following the development of the V-containing amino acids for peptide synthesis, we envisaged two main modifications on the previously discussed synthetic route: (a) replacement of the 1-(carboxymethyl)pyridin-1-ium moiety for a more stable acid functionality (i.e., 1-(4-carboxybenzyl)pyridin-1-ium),¹⁸ able to surpass potential decomposition by decarboxylation (*vide supra*)²⁰ and (b) the introduction of suitable amino protecting groups within our V-amino acid hybrids.²³ Consequently, we decided to tackle first the obtention of compound Boc-4·2Cl, a *tert*-butylcarbonyl (Boc)-*N*-protected derivative suitably protected for LPPS. In this case, the V-amino acid hybrid was synthesized in a good 49% overall yield, first by the Zincke reaction between readily available *N*-Boc-ethylene diamine and the 2,4-dinitrobenzene-activated salt of B_p ,¹⁹ followed by a subsequent alkylation of intermediate 3·Cl with 4-(chloromethyl)benzoic acid (Scheme 2).

With the *N*-protected amino acid Boc-4·2Cl in our hands, we proceeded to assess its use on LPPS by addressing the preparation of the simple model tripeptide Fmoc-L-Phe-4-D-Phe-OMe-2TFA (7·2TFA). The synthesis of this compound was performed from the C- to N-terminus so, first, Boc-4·2Cl was coupled to the D-phenylalanine methyl ester to give the dipeptide 5·2Cl. Next, cleavage of the Boc group with TFA resulted in the ammonium salt 6·3TFA, which was subsequently used without further purification on the final coupling with Fmoc-L-Phe-OH leading to 7·2TFA. The compound was obtained on a decent 35% overall yield, with

Scheme 2. Synthesis of the *N*-Protected V–Amino Acid Hybrid Boc-4·2Cl and Its Implementation on the Synthesis of the Model Tripeptide 7·2TFA



an analytical sample being purified by HPLC, and extensively characterized by ESI-MS spectrometry and NMR spectroscopy (Figure 3).

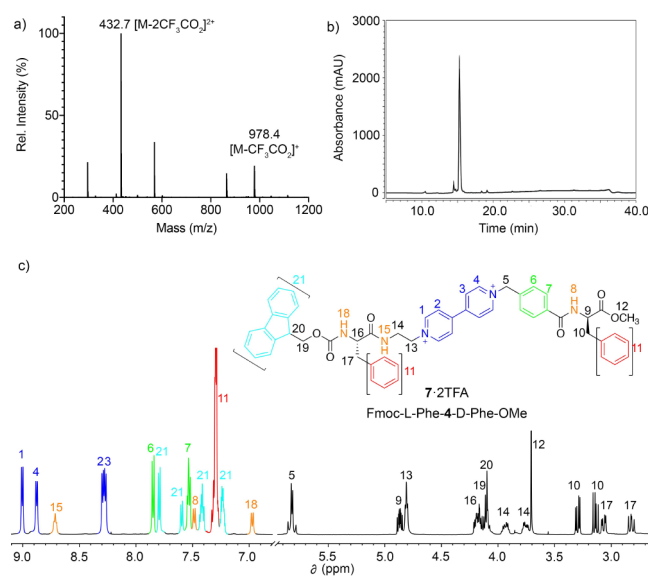


Figure 3. (a) MS spectrum corresponding to the peak at $t_R = 16$ min of the HPLC chromatogram at 220 nm (b) for 7·2TFA. (c) Partial ¹H NMR spectra (CD₃CN, 500 MHz) for 7·2TFA including the assignment based on 1D and 2D NMR.

Among other features, the assignment of the ¹H NMR spectrum of 7·2TFA allows us to identify in the aromatic region not only distinctive resonances attributable to the benzyl, phenylene, and Fmoc moieties but also the other four characteristic doublets of the V core. Regarding the ESI-MS, the spectrogram shows the signal attributable to the 7²⁺ cation as the base peak at *m/z* calcd for $[M - 2CF_3CO_2]^{2+}$ 432.6914, found 432.6915.

Finally, we tackled the implementation of our V–amino acid hybrids into SPPS. For this purpose, we devised first the synthesis of the appropriate *N*-protected amino acid Alloc-4·2Cl, having an Alloc group, orthogonal to Fmoc, and that would enable the classic Fmoc/*t*-Bu SPPS strategy.²⁴ To obtain Alloc-4·2Cl, we used a slight modification of the synthetic protocol explained above for Boc-4·2Cl (Scheme 1), with the introduction of the protecting group on intermediate

1H·2Br just before the second alkylation of B_p (Figure 4a). In that manner, the targeted amino acid was successfully

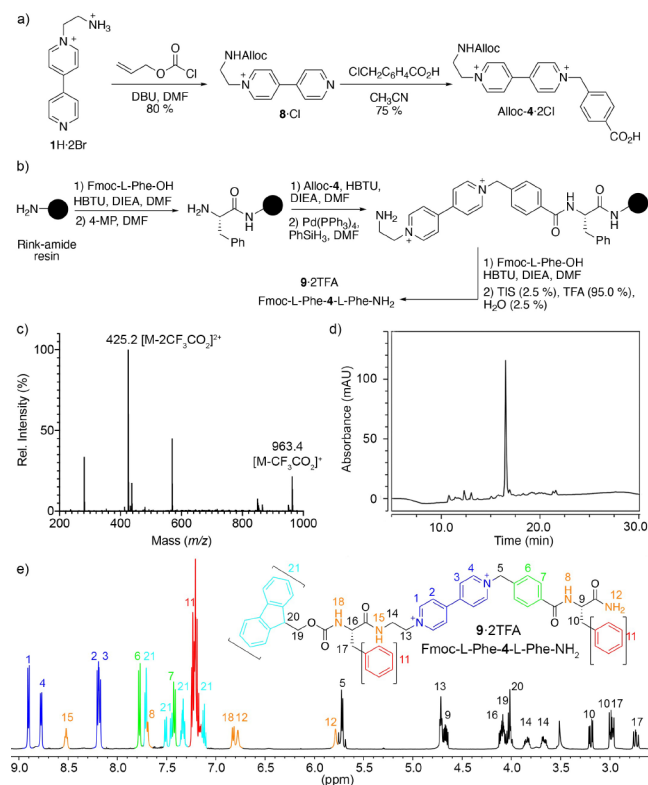


Figure 4. (a) Synthesis of the *N*-Alloc protected V–amino acid hybrid Alloc-4·2Cl. (b) SPPS of the model tripeptide 9·2TFA. (c) MS spectrum corresponding to the peak at $t_R = 16.6$ min of the HPLC chromatogram at 220 nm (d) for 9·2TFA. (e) Partial ¹H NMR spectra (CD₃CN, 500 MHz) for 9·2TFA including the assignment based on 1D and 2D NMR data.

synthesized in an excellent 51% overall yield, simply by reaction of the aminoethylbipyridium 1H·2Br with allyl chloroformate followed by the introduction of the corresponding 4-(chloromethyl)benzoic acid moiety. Consequently, by using the Fmoc/*t*-Bu strategy on a Rink amide resin, we followed with the SPPS of the model tripeptide Fmoc-L-Phe-4-L-Phe-NH₂ (9·2TFA). As shown in Figure 4b, that was achieved by coupling of Fmoc-L-Phe-OH to the resin and subsequent deprotection, followed by introduction of the V moiety by coupling of Alloc-4·2Cl using HBTU/DIEA in DMF. Alloc deprotection using a slight modification of the classic Pd(PPh₃)₄/PhSiH₃ method,²⁵ immediately followed by the coupling of Fmoc-L-Phe-OH and cleavage from the solid support, led to tripeptide 9·2TFA on a 6% overall yield after semipreparative HPLC purification. The associated ESI-MS spectrogram for the main peak on the HPLC chromatogram showed peaks at *m/z* = 963.3699 and *m/z* = 425.1915 corresponding to the loss of the trifluoroacetate anions on the expected structure (Figure 4c,d). Furthermore, the 1D/2D NMR experiments recorded in CD₃CN/D₂O for the purified reaction product showed data fully consistent with that observed for 7·2TFA and expected for 9·2TFA and allowed for a full assignment of the ¹H/¹³C nuclei in the molecule. As in the case of 7·2TFA, the introduction of the V moiety within the analogous tripeptide 9 can be easily inferred from the

diagnostic resonances of the electroactive unit on the ^1H NMR (Figure 4e).

Finally, we tried to qualitatively assess the interaction between one of our model peptides (7·2TFA) and CB[8]. Hence, a 1 mM solution of 7·2TFA, in D_2O with 50 mM phosphate buffer solution at $\text{pD} = 7$, was saturated with CB[8] and the corresponding ^1H NMR recorded after filtration of excess nondissolved macrocycle.²² Although the resulting complex pattern of broadened signals qualitatively imply an interaction between CB[8] and the amino acid–viologen hybrid, further extensive investigation would be needed in order to properly characterize this intricate system.¹⁷

In summary, we have described in this work the development of a series of viologen–amino acid hybrids that have been efficiently prepared both as “naked” or as *N*-protected derivatives suitable for L/SPPS. The ability of obtained amino acids as typical first guests of the cucurbituril family of hosts was explored for the unprotected derivative 2H·3Cl, being found to behave in a similar fashion with both CB[7] and CB[8], as other simple viologen derivatives. Furthermore, we have corroborated the implementation of the *N*-protected derivatives Boc·4·2Cl and Alloc·4·2Cl on, respectively, the L and SPPS of a model tripeptide having the Phe-V-Phe sequence. Overall, these results expand considerably not only the toolbox for the synthesis of new (redox) stimuli-responsive peptide materials,²⁶ but they open as well the door for the transient noncovalent modification of peptide structures by means of CB[8]-based heteroternary complexation,²⁷ an area that we are currently exploring in our laboratories.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.joc.1c02040>.

Experimental procedures, characterization data for new compounds, and titration experiments for CB[7,8]-complexes (PDF)

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All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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