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Synthesis of Non-Natural Aromatic α-Amino Acids by a Heck Reaction

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Alkene **3**, a TBS- and Cbz-protected derivative of vinylglycinol, safely available from methionine, undergoes efficient Heck coupling reactions in high yields. The resulting products can be converted into enantiopure α-amino acids with aromatic and heteroaromatic side-chains. Compound **6a**, a brightly fluorescent Fmoc-protected pyrene amino acid is of special interest. Tripeptide **8a**, composed of this building block and two arginines, binds to TAR, an RNA element regulating transcriptional efficiency in HIV, with a K_d value of 50 nM. This compound also inhibits a coupled cell-free transcription-translation assay (IC_{50} = 40 μM) and shows pronounced antibacterial activity against *B. subtilis*.

Introduction

RNA with its various secondary and tertiary structures offers binding sites for proteins and also for antibiotics and other small molecules. Therefore, RNA has become an interesting drug target in the last few years.[1] Recently, we reported on cationic tripeptides that bind to the viral RNA element TAR derived from HIV-1.[2] RNA complexation by these tripeptides disrupts the Tat-TAR complex, a molecular switch that regulates transcriptional efficiency in HIV,[3,4] and ultimately blocks the spread of HIV in cell culture experiments.[2] Non-covalent interactions between RNA and ligand molecules, for example, peptides, are governed by hydrogen bonds, charge–charge attraction and stacking. Thus, the TAR-binding tripeptides were composed of arginine and synthetic amino acids characterised by aromatic and heteroaromatic side-chains. Such residues are able to participate in stacking and to form hydrogen bonds.

Various methods exist for the synthesis of non-natural amino acids.[5] Enantioselective transformations of prochiral starting materials have given excellent results. However, such methods require the synthetic procedure for each new amino acid to be optimised individually and for the enantiomeric purity of every single batch to be checked. To avoid these problems, we had chosen a different strategy that relies on racemisation-proof transformations of a central enantiopure intermediate.[6] Derivatives of allylglycine[7] or vinylglycinol[8] fulfil such criteria. Compound **3** for example, after hydroboration of its alkene moiety, reacts with a broad range of aryl and heteroaryl bromides in a Suzuki cross-coupling reaction.[6] Herein we report on the Heck reactions of vinylglycinol **3** for the attachment of aromatic side-chains. The resulting products were converted into amino acids and finally into RNA-binding tripeptides of the general structure H_2N-(D)-Arg-X-(D)-Arg-$CONH_2$. The synthesis of a different vinylglycine derivative from serine via the Garner aldehyde has already been reported.[9] Such alkenes are known to be suitable substrates for Heck cross-coupling reactions;[10] however, owing to the high racemisation risk of the Garner aldehyde, we preferred an alternative method starting from methionine **1** which can be converted into vinylglycinol **3**.

Results and Discussion

Heck Reactions of Vinylglycinol 3

Previously we have used $LiAlH_4$ for the reduction of (L)-methionine (**1**) to methioninol (**2**) (76%, Scheme 1). However, almost quantitative yields were obtained when $LiAlH_4$ was replaced by the safer reagent $LiBH_4$.[11] All subsequent steps to convert **2** into vinylglycinol **3** by a thermal *syn* elimination reaction were conducted as reported before.[6]

The Heck coupling of aryl bromides and vinylglycinol **3** proved troublesome in the first experiments and required extensive optimisation until satisfying yields were achieved (Table 1). Finally, reaction conditions involving the use of quaternary ammonium salts[12] as promoter and $Pd(OAc)_2$ as catalyst without the addition of further ligands were

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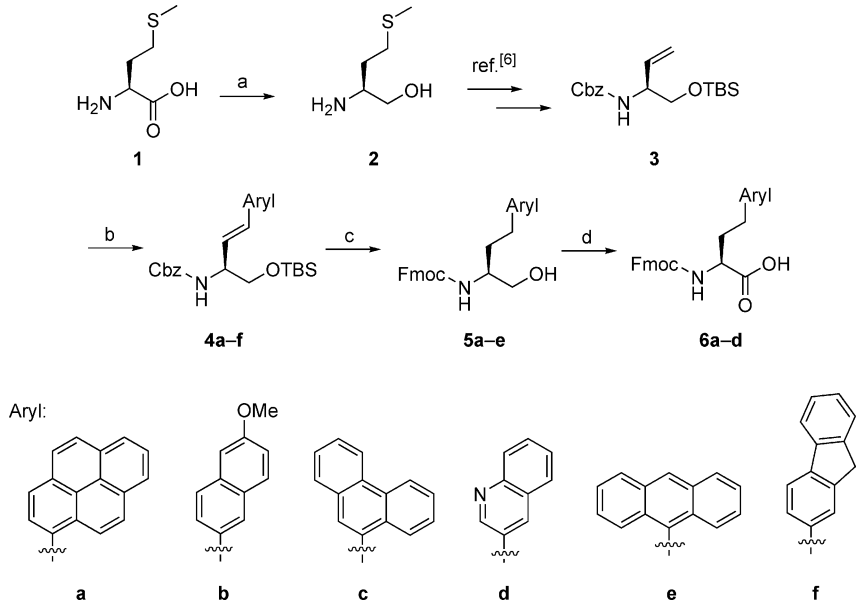


Scheme 1. Reagents: (a) $LiBH_4$, TMSCl, THF; (b) aryl-Br, $Pd(OAc)_2$, K_2CO_3, Bu_4NOTf, DMF/H_2O; (c) (1) 1,4-cyclohexadiene, $Pd(OH)_2$ or H_2, Pd/C for **4b**, (2) TBAF, (3) Fmoc-OSu; (d) PDC, DMF.

found to be optimal (DMF/water, 100 °C, 16 h). Yields of the Heck coupling products **4a**–**e** above 75%, depending on the steric hindrance of the aromatic system, were reproducibly obtained. The cross coupling of **3** with 2-bromofluorene was an exception. In this case quite different conditions were required. In view of the low yield of **4f** (30%), we did not try to convert this compound into the corresponding amino acid. The large coupling constants of the vinyl hydrogen signals observed in the ^1H NMR spectra of compounds **4b**, **4d** and **4f** unequivocally proved the clean (*E*) configuration of the alkene moieties. It is thus reasonable to assume the same stereochemistry for the other Heck products as well. In all the coupling experiments leading to alkenes **4a**–**e**, the corresponding (*Z*) isomers could neither be isolated nor detected by NMR spectroscopy.

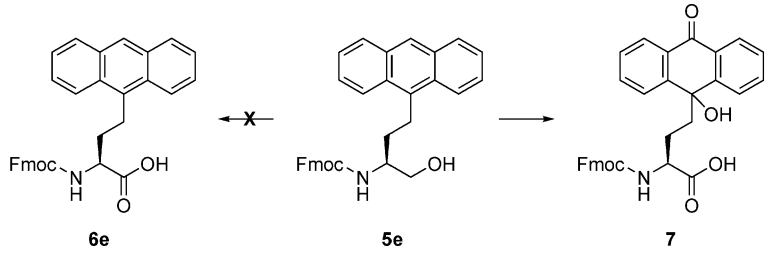
Table 1. Reaction conditions and yields.

	Yield [%]					
	a	**b**	**c**	**d**	**e**	**f**
4	87[a]	89[a]	83[a]	80[a]	77[a]	30[b]
5	59[c]	50[d]	76[c]	73[c]	58[c]	–
6	64[e]	71[e]	80[e]	73[f]	–	–

[a] $Pd(OAc)_2$, K_2CO_3, Bu_4NOTf, DMF/water (20:1), 100 °C. [b] $Pd(OAc)_2$, K_2CO_3, Bu_4NCl, DMF, 100 °C. [c] (1) $Pd(OH)_2$, 1,4-cyclohexadiene, reflux, (2) TBAF, (3) FmocOSu. [d] (1) H_2, Pd/C, (2) TBAF, (3) FmocOSu. [e] PDC, DMF. [f] PDC, DMF, few drops of H_2SO_4.

Having introduced the aromatic side-chains, the protecting groups were removed and the styrene moiety reduced. Standard hydrogenolysis conditions (H_2, Pd/C), however, were only successful for the naphthalene derivative **4b**. To avoid a partial reduction of the aromatic side-chain, we applied a transfer hydrogenation protocol with 1,4-cyclohexadiene and $Pd(OH)_2$ in refluxing ethanol in all other cases.[13] Subsequent to the desilylation with TBAF in THF, Fmoc protection of the amino group was carried out (**5a**–**e**). The final step to complete the synthesis of the amino acid building blocks **6a**–**d** is the oxidation of the protected amino alcohols **5a**–**d** with PDC in DMF.[14] This oxidation procedure, however, failed to convert the anthracene alcohol **5e** into amino acid **6e**. Apart from oxidation of the alcohol, oxidative transformation of the aromatic system into hydroxyanthrone **7** occurred (Scheme 2).

To rule out racemisation in the Heck coupling step, we also prepared *ent*-**3** from (D)-methionine and converted it into *ent*-**4a**. The availability of the two enantiomers **4a** and *ent*-**4a** allowed us to demonstrate an optical purity of >99% by chiral HPLC. As shown previously, the subsequent protection and oxidation steps can be regarded



Scheme 2. Oxidation of the anthracene amino alcohol **5e** with PDC in DMF.

as racemisation-proof.[6] We therefore conclude that the method shown in Scheme 1 allows a stereochemically safe transformation of methionine into Fmoc-protected amino acid building blocks.

Application to Solid-Phase Peptide Synthesis

Each Fmoc-protected building block **6a**–**d** was converted into tripeptides **8a**–**d** (Figure 1). Arginine was chosen because of its crucial role in Tat-TAR binding. Whereas compounds **8b**–**d** are identical in all aspects to material prepared previously by the Suzuki coupling approach,[6] the brightly fluorescent pyrene peptide **8a** is a new compound that exhibits interesting biological activities, as shown below.

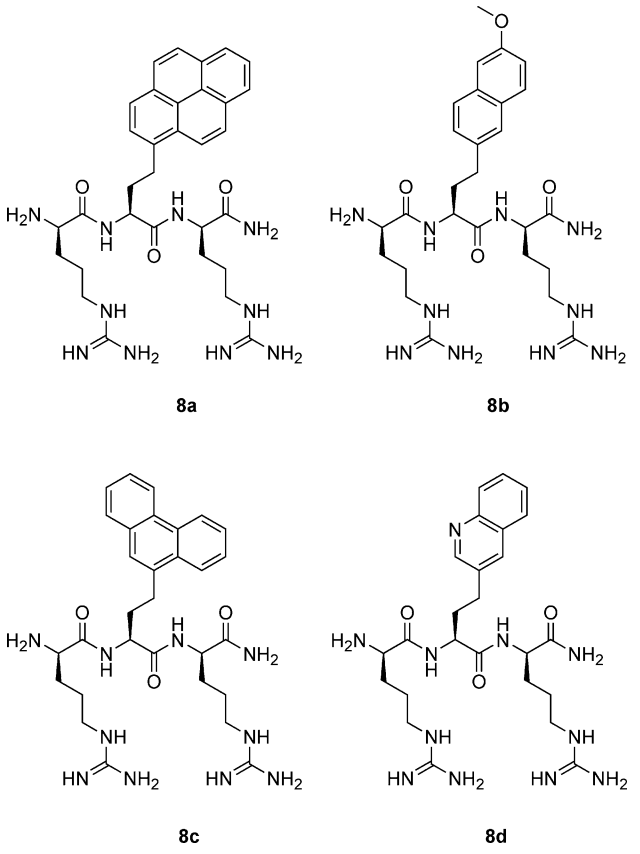


Figure 1. Tripeptides generated from the building blocks **8a**–**d** by standard Fmoc solid-phase synthesis.[15] The non-protonated form is shown.

RNA Binding Assays

A fluorimetric competition assay was used to determine the RNA binding affinities of compounds **8a**–**d** (Figure 2).[2,16] Tat peptide **9**, labelled with fluorescein and rhodamine, has a flexible structure and shows efficient quenching of the dyes when RNA is absent. On binding to TAR RNA **10**, a 2.6-fold fluorescence gain was observed as a result of conformational changes in peptide **9**. Titrations with compounds **8a**–**d** as competitors replace peptide **9** from the Tat binding site of **10**, thus restoring the low initial fluorescence. This allows measurement of the IC_{50} values of peptides **8a**–**d** (Table 2).

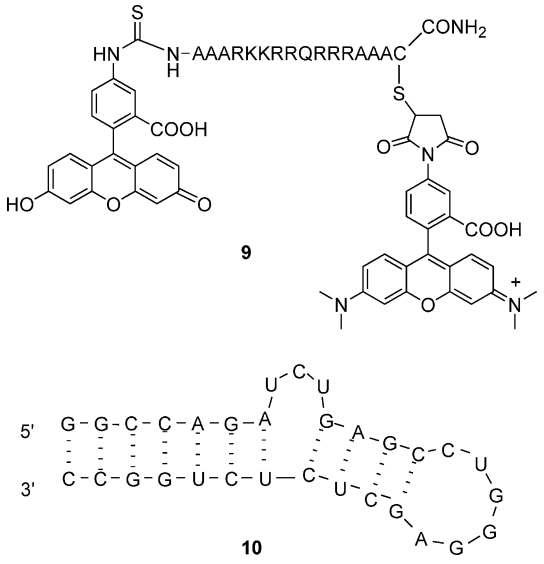


Figure 2. Structures of the Tat peptide **9** and the HIV-1 TAR RNA model **10**.

Table 2. TAR affinities of the non-natural peptides **8a**–**d**.[a]

Peptide	IC_{50} [μM]	K_d [nM]
8a	0.9	50
8b	15[b]	–
8c	3[b]	–
8d	30[b]	–

[a] Experimental error: ± 30%. [b] Ref.[2]

Alternatively, the pyrene moiety of **8a** allows the direct determination of K_d for this peptide.[17] The fluorescence intensity is largely reduced upon binding to RNA **10**. Fitting of a 1:1 complexation model to the titration curve (Figure 3) using a non-linear least-squares calculation with K_d as a variable leads to the binding constant (Table 2). The data characterise tripeptide **8a** as one of the most potent small-molecule ligands for TAR known today.[18,19]

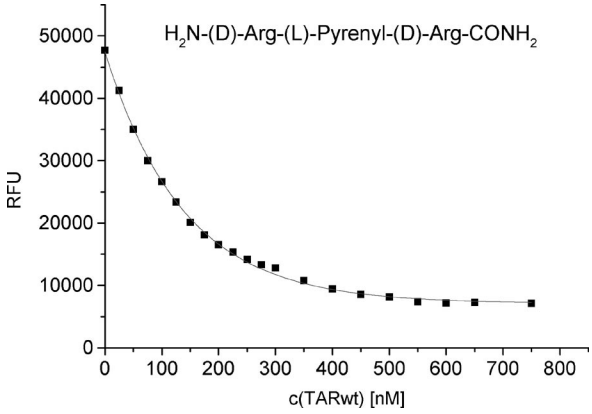


Figure 3. Fluorescence titration of peptide **8a** with TAR **10**. Assay conditions: 100 nM pyrene peptide **8a**, 50 mM Tris-HCl, pH = 7.4, 20 mM KCl, 0.01% Triton-X100; λ_{ex} = 340 nm, λ_{em} = 400 nm. The solid line shows the best fit of a 1:1 binding model to the experimental data points.

Antibacterial Properties of Peptide 8a

Binding to different parts of bacterial ribosomes is the mode of action for numerous antibiotics. Since the target specificity of RNA ligands is limited in general, tripeptide **8a** was also regarded as a candidate structure to block bacterial translation. This assumption was tested in a cell-free coupled transcription-translation assay expressing green fluorescent protein GFP. The IC_{50} value determined for **8a** is 36 ± 3 μM. For comparison, IC_{50} values of 37 and 2 μM were found in this assay for chloramphenicol and erythromycin, two widely used translation inhibitors.[20] The IC_{50} value against *Bacillus subtilis 168* was determined to be 20 ± 2 μM by the microtitre broth dilution method.[21] The corresponding numbers for erythromycin and chloramphenicol, determined against the pathogenic bacteria *B. anthracis* and *B. cereus*, are 0.7 and 12 μM, respectively.[22] Thus, with respect to antibacterial potency, peptide **8a** is inferior to erythromycin but comes close to chloramphenicol in this initial assay.

Conclusions

With the improved reduction method for methionine, vinylglycinol **3** is safely accessible on a large scale. To attach aromatic and heteroaromatic rings, the vinyl group may either react by the Heck procedure, as shown above, or it may be hydroborated and cross-coupled in a Suzuki reaction.[6] Hydroboration of **3** with 9-BBN requires elevated temperatures in 1,4-dioxane. This critical step, which can be a source of failure, is no longer necessary in the Heck procedure. On the other hand, yields of the Suzuki methods when performed under optimal conditions slightly surpassed the yields of the Heck reaction described herein. In the latter method, the alkenyl group of intermediates **4a**–**f** has to be reduced. However, this does not require an additional step as the double bond is automatically reduced during Cbz removal by transfer hydrogenation. Both procedures ultimately deliver Fmoc-protected enantiopure amino acids. The Heck and Suzuki procedures therefore can be considered equivalent. With certain substrates, however, each method may offer specific advantages. The new pyrene amino acid **6a** is of special interest. Its large aromatic framework is perfectly suited to stacking with nucleobases and base pairs. Furthermore it represents a versatile fluorescent probe for investigating the interaction with all kinds of binding partners, including RNA. In the Tat-TAR competition assay, the pyrene peptide **8a** exhibited the best IC_{50} value that we have so far observed with tripeptides.[2] Subsequent studies will investigate the antiviral properties of **8a** in cell cultures and also the mechanism of its antibiotic action.

Experimental Section

General: NMR: Bruker DPX 250 (^{1}H: 250 MHz; ^{13}C: 62.9 MHz), Bruker AM 300 (^{1}H: 300 MHz; ^{13}C: 75.4 MHz) or Bruker Avance 400 (^{1}H: 400 MHz; ^{13}C: 100.6 MHz). FTIR: Perkin-Elmer 1600 Series or Jasco 420. Elemental analysis: Heraeus CHN Rapid. Mass spectrometry: Fisons VG Platform II (ESI) and Fisons VG Tofspec (MALDI). Melting points (uncorrected): Kofler hot-plate microscope. Optical rotation: Perkin-Elmer polarimeter 241.

General Procedure for the Heck Cross-Coupling Products 4a–e: Vinyl compound **3** (500 mg, 1.49 mmol) was dissolved in DMF (20 mL) and water (1 mL); the mixture was degassed and flushed with argon. Then K_2CO_3 (500 mg, 3.60 mmol), Bu_4NOTf (710 mg, 1.8 mmol), aryl halide (1.80 mmol) and $Pd(OAc)_2$ (33 mg, 0.15 mmol) were added. The solution was degassed once again. After flushing with argon, the reaction mixture was stirred at 100 °C for 16 h. The reaction was quenched with brine and the mixture filtered through Celite®. The filtrate was extracted three times with EtOAc. The combined organic phases were dried with $MgSO_4$ and concentrated in vacuo.

Benzyl (*S*)-[1-(*tert*-Butyldimethylsilyloxymethyl)-3-(pyren-1-yl)allyl]-carbamate (4a): The residue was purified by column chromatography (*n*-hexane/EtOAc, 25:1, then 10:1). After recrystallisation from MeCN, a colourless solid (710 mg, 87%) was obtained. R_f = 0.2 (*n*-hexane/EtOAc, 10:1). M.p. 101–103 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.48 (d, J = 9.5 Hz, 1 H, aryl-*H*), 8.30–8.14 (m, 7 H, aryl-*H*), 8.06 (m, 1 H, aryl-*H*), 7.71–7.65 (m, 2 H, aryl-C*H*, N*H*, exchangeable with D_2O), 7.43–7.31 (m, 5 H, C_6H_5), 6.51 (dd, J = 15.8, J = 6 Hz, 1 H, aryl-CH=C*H*), 5.12 (s, 2 H, Ph-CH_2), 4.53 (m, 1 H, NC*H*), 3.75 (m, 2 H, CH_2-OTBS), 0.86 [s, 9 H, (CH_3)$_3$], 0.06 [s, 6 H, (CH_3)$_2$] ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 155.8, 137.1, 131.5, 131.2, 130.9, 130.3, 130.1, 128.2, 127.69, 127.65, 127.4, 127.3, 127.1, 127.0, 126.2, 125.2, 125.1, 125.0, 124.0, 123.9, 123.5, 123.0, 65.3, 65.1, 55.1, 25.7, 17.8, –5.4 ppm. IR (KBr): $\tilde{\nu}$ = 3295 (m), 3037 (m), 2929 (m), 1688 (s), 1544 (s), 1462 (w), 1279 (m), 1247 (m), 1115 (m), 1041 (m), 961 (w), 839 (s), 776 (m) cm^{-1}. $C_{34}H_{37}NO_3Si$ (535.75): C 76.22, H 6.96, N 2.61; found C 76.38, H 6.96, N 2.38. $[\alpha]_D^{20}$ = +4.6 (c = 0.5, MeOH); *ent*-**4a**: $[\alpha]_{20}^{D}$ = –4.6 (c = 0.5, MeOH). The optical purity was determined to be >99% by HPLC analysis (Chiral Whelk RR column, *n*-hexane/*i*PrOH, 1:1, 1.0 mL/min, 254 nm): $t_R(R)$ = 9.58 min; $t_R(S)$ = 21.67 min.

Benzyl (*S*)-[1-(*tert*-Butyldimethylsilyloxymethyl)-3-(6-methoxynaphthalen-2-yl)allyl]carbamate (4b): The residue was purified by column chromatography (*n*-hexane/EtOAc, 25:1, then 10:1). After recrystallisation from MeCN, a colourless solid (653 mg, 89%) was obtained. R_f = 0.15 (*n*-hexane/EtOAc, 10:1). M.p. 66–67 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 7.76 (m, 3 H, aryl-*H*), 7.57 (d, J = 8.5 Hz, 1 H, aryl-*H*), 7.45 (d, J = 8.5 Hz, 1 H, aryl-*H*), 7.33 (m, 5 H, C_6H_5), 7.29 (d, J = 2.25 Hz, N*H*, exchangeable with D_2O), 7.15 (dd, J = 9, J = 2.5 Hz, 1 H, aryl-*H*), 6.65 (d, J = 16 Hz, 1 H, aryl-C*H*=CH), 6.29 (dd, J = 16, J = 6.25 Hz, 1 H, aryl-CH=C*H*), 5.06 (s, 2 H, Ph-CH_2), 4.31 (m, 1 H, NC*H*), 3.86 (s, 3 H, OCH_3), 3.65 (m, 2 H, CH_2-OTBS), 0.85 [s, 9 H, (CH_3)$_3$], 0.04 [s, 6 H, (CH_3)$_2$] ppm. ^{13}C NMR (62.9 MHz, [D$_6$]DMSO): δ = 157.3, 137.1, 133.7, 131.7, 130.4, 129.4, 128.4, 128.2, 127.7, 127.6, 127.2, 127.0, 125.7, 123.7, 118.7, 105.9, 65.2, 55.1, 25.6, 17.9, –5.4 ppm. IR (KBr): $\tilde{\nu}$ = 3363 (m), 3316 (m), 3036 (w), 2954 (m), 2855 (m), 1688 (s), 1630 (m), 1602 (m), 1534 (s), 1389 (m), 1279 (s), 1242 (s), 1122 (s), 1040 (s), 975 (m), 900 (w), 838 (s), 777 (m), 670 (w) cm^{-1}. $C_{29}H_{37}NO_4Si$ (491.69): C 70.84, H 7.58, N 2.85; found C 70.83, H 7.84, N 3.09. $[\alpha]_{20}^{D}$ = +34.9 (c = 1.0, MeOH).

Benzyl (*S*)-[1-(*tert*-Butyldimethylsilyloxymethyl)-3-(phenanthren-9-yl)allyl]carbamate (4c): The residue was purified by column chromatography (*n*-hexane/EtOAc, 25:1, then 10:1). After recrystallisation from MeCN, a colourless solid (630 mg, 83%) was obtained. R_f = 0.25 (*n*-hexane/EtOAc, 10:1). M.p. 76 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.85 (d, J = 7.5 Hz, 1 H, aryl-*H*), 8.78

(m, 1 H, aryl-*H*), 8.22 (d, *J* = 7.8 Hz, 1 H, aryl-*H*), 7.97 (m, 1 H, aryl-*H*), 7.88 (s, 1 H, aryl-*H*), 7.75–7.63 (m, 5 H, aryl-*H*, N*H*, exchangeable with D_2O), 7.42–7.33 (m, 6 H, C_6H_5, aryl-C*H*), 6.34 (dd, *J* = 15.8, *J* = 6 Hz, 1 H, aryl-CH=C*H*), 5.11 (s, 2 H, Ph-CH_2), 4.47 (m, 1 H, NC*H*), 3.73 (m, 2 H, CH_2-OTBS), 0.87 [s, 9 H, (CH_3)$_3$], 0.06 [s, 6 H, (CH_3)$_2$] ppm. ^{13}C NMR (62.9 MHz, [D$_6$]-DMSO): δ = 155.7, 137.1, 133.1, 131.5, 131.2, 129.8, 129.7, 129.4, 128.4, 128.2, 127.7, 126.9, 126.7, 126.6, 124.5, 123.9, 123.2, 122.6, 65.3, 65.1, 54.8, 25.6, 17.8, –5.4 ppm. IR (KBr): $\tilde{\nu}$ = 3305 (m), 3063 (w), 2953 (m), 2858 (m), 1690 (s), 1541 (s), 1460 (w), 1288 (m), 1250 (m), 1120 (m), 1052 (m), 964 (w), 842 (m), 776 (m), 745 (m), 695 (w) cm^{-1}. $C_{32}H_{37}NO_3Si$ (511.73): C 75.11, H 7.29, N 2.74; found C 74.89, H 7.35, N 2.58. $[\alpha]_{20}^{D}$ = +29.2 (*c* = 1.0, MeOH).

Benzyl (*S*)-[1-(*tert*-Butyldimethylsilyloxymethyl)-3-(quinolin-3-yl)-allyl]carbamate (4d): The residue was purified by column chromatography (*n*-hexane/EtOAc, 10:1, then 3:1). A colourless oil (552 mg, 80%) was furnished after drying in vacuo. R_f = 0.45 (*n*-hexane/EtOAc, 2:1). ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 9.00 (d, *J* = 2.3 Hz, 1 H, aryl-*H*), 8.29 (s, 1 H, aryl-*H*), 7.97 (m, 2 H, aryl-*H*), 7.72 (m, 1 H, aryl-*H*), 7.60 (m, 1 H, aryl-*H*), 7.50 (d, *J* = 8.3 Hz, 1 H, N*H*, exchangeable with D_2O), 7.37–7.31 (m, 5 H, C_6H_5), 6.74 (d, *J* = 16.3 Hz, 1 H, aryl-C*H*=CH), 6.54 (dd, *J* = 16.3, *J* = 5.8 Hz, 1 H, aryl-CH=C*H*), 5.07 (s, 2 H, Ph-CH_2), 4.35 (m, 1 H, NC*H*), 3.67 (m, 2 H, CH_2-OTBS), 0.85 [s, 9 H, (CH_3)$_3$], 0.05 [s, 6 H, (CH_3)$_2$] ppm. ^{13}C NMR (100.6 MHz, [D$_6$]DMSO): δ = 155.8, 149.0, 146.8, 137.1, 132.0, 130.6, 129.5, 129.2, 128.6, 128.3, 128.1, 127.7, 127.6, 127.2, 127.0, 126.3, 65.3, 64.8, 54.9, 25.7, 17.9, –5.4 ppm. IR (film): $\tilde{\nu}$ = 3440 (m), 3324 (m), 3033 (m), 2953 (s), 2856 (s), 1718 (s), 1495 (s), 1470 (m), 1375 (w), 1253 (s), 1112 (s), 968 (m), 909 (w), 837 (m), 780 (m), 697 (m) cm^{-1}. $C_{27}H_{34}N_2O_3Si$ (462.66): C 70.09, H 7.41, N 6.05; found C 69.94, H 7.44, N 6.17. $[\alpha]_{20}^{D}$ = +29.1 (*c* = 0.8, MeOH).

Benzyl (*S*)-[3-(Anthracen-9-yl)-1-(*tert*-butyldimethylsilyloxymethyl)-allyl]carbamate (4e): The residue was purified by column chromatography (*n*-hexane/EtOAc, 25:1). After recrystallisation from MeCN, a light yellow solid (590 mg, 77%) was obtained. R_f = 0.2 (*n*-hexane/EtOAc, 10:1). M.p. 68–70 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.54 (s, 1 H, aryl-*H*), 8.29 (d, *J* = 7.8 Hz, 2 H, aryl-*H*), 8.09 (dd, *J* = 7.3, *J* = 2 Hz, 2 H, aryl-*H*), 7.70 (d, *J* = 7.5 Hz, 1 H, N*H*, exchangeable with D_2O), 7.56–7.28 (m, 10 H, C_6H_5, aryl-C*H*, aryl-*H*), 5.97 (dd, *J* = 16.3, *J* = 6 Hz, 1 H, aryl-CH=C*H*), 5.12 (s, 2 H, Ph-CH_2), 4.54 (m, 1 H, NC*H*), 3.80 (m, 2 H, CH_2-OTBS), 0.89 [s, 9 H, (CH_3)$_3$], 0.10 [s, 6 H, (CH_3)$_2$] ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO): δ = 155.8, 137.1, 136.4, 132.1, 130.8, 128.7, 128.4, 128.3, 127.70, 127.67, 126.1, 125.9, 125.6, 125.4, 125.2, 65.3, 64.9, 55.1, 25.7, 17.9, –5.4 ppm. IR (KBr): $\tilde{\nu}$ = 3330 (m), 2932 (m), 2859 (m), 1943 (w), 1712 (s), 1503 (s), 1335 (w), 1257 (m), 1105 (m), 833 (m), 775 (m), 733 (m) cm^{-1}. $C_{32}H_{37}NO_3Si$ (511.73): C 75.11, H 7.29, N 2.74; found C 75.02, H 7.19, N 2.58. $[\alpha]_{20}^{D}$ = +7.9 (*c* = 0.95, MeOH).

Benzyl (*S*)-[1-(*tert*-Butyldimethylsilyloxymethyl)-3-(9*H*-fluoren-2-yl)allyl]carbamate (4f): Vinyl compound **3** (500 mg, 1.49 mmol) was dissolved in dry DMF (10 mL) and degassed. Then, molecular sieves (3 Å) (500 mg), K_2CO_3 (500 mg, 3.60 mmol), Bu_4NCl (500 mg, 1.80 mmol), 2-bromofluorene (370 mg, 1.49 mmol) and $Pd(OAc)_2$ (33 mg, 0.15 mmol) were added to the solution. The mixture was degassed, flushed with argon and stirred at 100 °C for 16 h. For workup, the reaction was quenched with brine and the mixture filtered through Celite®. The filtrate was extracted three times with EtOAc. The combined organic phases were dried with $MgSO_4$ and the solvents evaporated in vacuo. The residue was purified by column chromatography (*n*-hexane/EtOAc, 10:1). After drying in vacuo, a yellow solid (230 mg, 30%) was obtained. R_f = 0.15 (*n*-hexane/EtOAc, 10:1). M.p. 86–87 °C. ^{1}H NMR (250 MHz, [D$_6$]-DMSO): δ = 7.85 (t, *J* = 6.5 Hz, 2 H, aryl-*H*), 7.57 (s + d, *J* = 9.3 Hz, 2 H, aryl-*H*), 7.44–7.27 (m, 9 H, aryl-*H*, N*H*), 6.61 (d, *J* = 16 Hz, 1 H, aryl-C*H*), 6.26 (dd, *J* = 16, *J* = 6.25 Hz, 1 H, aryl-CH=C*H*), 5.05 (s, 2 H, Ph-CH_2), 4.28 (m, 1 H, NC*H*), 3.91 (s, 2 H, fluorenyl-9*H*), 3.63 (m, 2 H, CH_2-OTBS), 0.86 [s, 9 H, (CH_3)$_3$], 0.04 [s, 6 H, (CH_3)$_2$] ppm. IR (KBr): $\tilde{\nu}$ = 3428 (w), 3290 (m), 3034 (w), 2929 (m), 2857 (m), 1711 (s), 1691 (s), 1542 (m), 1459 (m), 1252 (m), 1083 (m), 969 (m), 838 (m), 738 (m) cm^{-1}. $C_{31}H_{37}NO_3Si$ (499.72): C 74.51, H 7.46, N 2.80; found C 74.58, H 7.52, N 3.03. $[\alpha]_{20}^{D}$ = –2.7 (*c* = 0.9, MeOH).

General Procedure for the Synthesis of the Fmoc-Protected Amino Alcohols 5a and 5c–e: The Heck coupling product **4a**,**c**–**e** (1 equiv.) was dissolved in EtOH (20 mL). 1,4-Cyclohexadiene (15 equiv.) and $Pd(OH)_2$ (0.3 equiv.) were added. The reaction mixture was refluxed for 16 h. After quantitative conversion, the mixture was filtered through Celite® and concentrated in vacuo. The crude product was dissolved in 1 M TBAF in THF (2.4 equiv.) and stirred at room temperature for at least 5 h. The reaction was quenched with saturated $NaHCO_3$ and the mixture extracted three times with EtOAc. The combined organic layers were dried with $MgSO_4$ and the solvents evaporated in vacuo. The residue was dissolved in EtOAc/EtOH (2:1, 30 mL) before Fmoc-OSu (1.1 equiv.) was added. Then the reaction mixture was stirred at room temp. for 1 h. The precipitate was filtered, washed with ice-cold EtOAc and dried in vacuo.

(9*H*-Fluoren-9-yl)methyl (*S*)-[1-(Hydroxymethyl)-3-(pyren-1-yl)propyl]carbamate (5a): Carbamate **4a** (500 mg, 0.93 mmol) was used as the starting material in this reaction. After recrystallisation from MeCN, a colourless solid (280 mg, 59%) was obtained. R_f = 0.35 (*n*-hexane/EtOAc, 1:1). M.p. 158–160 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.43–7.77 (m, 13 H, aryl-*H*), 7.44–7.30 (m, 5 H, aryl-*H*, N*H*, exchangeable with D_2O), 4.70 (t, *J* = 5.5 Hz, 1 H, O*H*, exchangeable with D_2O), 4.45–4.25 (m, 3 H, COOCH_2-C*H*), 3.62 (m, 1 H, NC*H*), 3.49–3.17 (m, 4 H, CH_2OH, aryl-CH_2), 2.02 (m, 1 H, aryl-CH$_2$-CH_a), 1.85 (m, 1 H, aryl-CH$_2$-CH_b) ppm. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO): δ = 157.1, 142.4, 139.3, 137.3, 137.0, 130.8, 130.3, 129.1, 128.8, 127.9, 127.34, 127.26, 127.2, 127.0, 126.3, 126.0, 124.85, 124.78, 124.6, 124.1, 124.0, 123.5, 121.3, 119.9, 109.6, 65.5, 52.5, 35.1, 29.1 ppm. IR (KBr): $\tilde{\nu}$ = 3446 (m), 3304 (s), 3037 (m), 2946 (m), 2870 (w), 1685 (s), 1546 (s), 1449 (m), 1354 (w), 1294 (m), 1267 (m), 1142 (w), 1056 (m), 842 (m), 738 (m), 624 (w) cm^{-1}. $C_{35}H_{29}NO_3$ (511.61): C 82.17, H 5.71, N 2.74; found C 81.97, H 5.95, N 2.97. $[\alpha]_{20}^{D}$ = +30.1 (*c* = 1.0, DMF).

(*S*)-2-[(9*H*-Fluoren-9-yl)methoxycarbonylamino]-4-(pyren-1-yl)-butyric Acid (6a): The Fmoc-protected amino alcohol **5a** (330 mg, 0.64 mmol) was dissolved in DMF (20 mL), and PDC (1.45 g, 3.87 mmol) was added to the clear solution. The mixture was stirred at room temp. overnight. For workup, the reaction was quenched with brine and the mixture extracted three times with EtOAc. The combined organic layers were washed with saturated aqueous $Na_2S_2O_5$ solution and concentrated in vacuo. The crude product was adsorbed on silica gel and purified by column chromatography (EtOAc to EtOAc/MeOH, 9:1). After recrystallisation from DCM/*n*-hexane, a colourless solid (217 mg, 64%) was obtained. R_f = 0.25 (EtOAc/MeOH, 9:1). M.p. 198–200 °C. ^{1}H NMR (250 MHz, [D$_6$]DMSO): δ = 8.39–7.64 (m, 14 H, aryl-*H*), 7.44–7.30 (m, 4 H, aryl-*H*, N*H*), 4.31 (m, 2 H, OCH_2), 4.05 (m, 1 H, OCH$_2$C*H*), 3.50–3.28 (m, 3 H, aryl-CH_2, NC*H*), 2.15 (m, 2 H, aryl-CH$_2$-CH_2) ppm; the resonance for COO*H* was not observed. ^{13}C NMR (75.4 MHz, [D$_6$]DMSO): δ = 155.9, 143.9, 143.7, 140.6,

139.3, 137.3, 136.1, 130.8, 130.3, 129.2, 128.8, 128.0, 127.5, 127.3, 127.2, 127.1, 127.0, 126.4, 126.0, 125.2, 124.8, 124.7, 124.1, 124.0, 123.3, 121.3, 120.0, 119.9, 109.6, 65.4, 54.4, 46.7, 33.6, 29.2 ppm. IR (KBr): $\tilde{\nu}$ = 3417 (s), 3040 (w), 2936 (w), 1700 (s), 1604 (m), 1511 (m), 1449 (m), 1418 (m), 1349 (m), 1247 (m), 1185 (w), 1053 (w), 845 (m), 740 (m), 621 (w) cm^{-1}. MS (ESI): *m*/*z* (%) = 524.3 (21.71) $[M - H]^-$, 328.1 (65.85) [M – H – fluorenylmethanol]$^-$, 302.0 (100) [M – H – Fmoc]$^-$. $[a]_{20}^{D}$ = +38.5 (*c* = 0.1, DMF).

General Procedure for Solid-Phase Peptide Synthesis: See Supporting Information.

H_2N-(D)-Arg-(L)-pyrenyl-(D)-Arg-$CONH_2$ (8a): Yield: 99%; HPLC conditions: preparative: Bischoff-Prontosil-H, 250 × 16, 0.1% TFA/MeCN (10:4), 7 mL/min; analytical: Reprosil AQ, 125 × 4.6, 0.1% TFA/MeCN (10:4), 0.8 mL/min, t_R = 4.47 min. MS (ESI): *m*/*z* (%) = 615.3 (37.11) $[M + H]^+$, 308.1 (100.0) $[M + 2H]^{2+}$.

Determination of K_d: Fluorescence-based binding assays were performed in 96-well microtitre plates (Corning 6860, black, non-binding surface) at 37 °C using a final volume of 100 μL in TK buffer (50 mM Tris-HCl, 20 mM KCl, 0.01% Triton-X100, pH = 7.4). 100 nM pyrene peptide **8a** was titrated against TAR RNA **10**. The following concentration range for the RNA **10** was chosen: 0–700 nM in steps of 25 nM. The blank contained only TK buffer. The fluorescence of the samples was measured with a fluorescence microplate reader (λ_{ex} = 340 nm, λ_{em} = 400 nm). Fluorescence readouts were corrected in relation to the blank value. A 1:1 binding model with K_d as a variable was then fitted to the experimental data points by a non-linear least-squares procedure. Each value of K_d was calculated from three independent experiments.

Determination of IC_{50}: Tat peptide **9** and TAR RNA **10** were both used at final concentrations of 10 nM. The fluorescence of pure peptide **9** and the Tat-TAR complex was determined first (Reader: Tecan safire^2; λ_{ex} = 540 nm, λ_{em} = 590 nm; 37 °C). Titration curves were determined from 11 data points. The competitor concentration at which the fitted titration curve intersected the mean fluorescence counts of the Tat-TAR complex and the uncomplexed Tat was taken as the IC_{50} value of the tripeptide.

Supporting Information (see also the footnote on the first page of this article): Synthetic procedures and characterisation data for all known compounds (**2**, **5b**–**e**, **6b**–**d**); general procedure for solid-phase peptide synthesis; descriptions of the coupled transcription-translation assay and of the antibacterial assay; ^1H and ^{13}C NMR spectra of **2**, **4a**–**f**, **5a**–**e**, and **6b**–**d**; chromatograms of **4a** and *ent*-**4a**.

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