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Silole amino acids with Aggregation-Induced Emission features synthesized by hydrosilylation reaction

Mathieu Arribat,[a] Emmanuelle Rémond,[a] Sébastien Richeter,[b] Philippe Gerbier,[b] Sébastien Clément,*[b] and Florine Cavelier*[a]

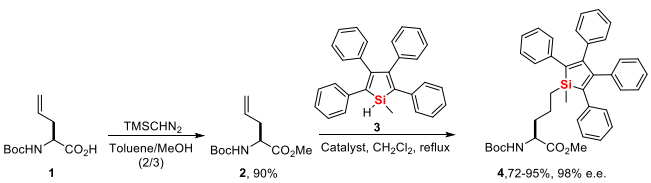
Abstract: The synthesis of silole amino acids was achieved through hydrosilylation of alkene or alkyne-containing amino acids with 1-methyl-2,3,4,5-tetraphenyl-1H-silole, using Karstedt's catalyst with yield up to 95% and without epimerization. After selective deprotection of carboxylic acid or amine functions respectively, C- or N-peptide coupling with an alanine moiety proved their possible incorporation into peptides. A model tripeptide was synthesized by solid phase synthesis with the N-Fmoc protected silole amino acid version. The silole moiety can be also grafted on a precursor peptide directly on the solid support. These amino acids and peptides exhibit AIE properties with λ_{em} ~500 nm and $\Delta\lambda$ ~100 nm. This approach constitutes an alternative and promising strategy for incorporation of such AIE fluorogens to peptides.

Introduction

Fluorescence technique has become an essential tool for biosensing, bioimaging and diagnosis by virtue of its high sensitivity, easy operation and non-invasiveness.[1] The continuous development of sophisticated optics and electronics associated to fluorescence requires the design of fluorophores with suitable features.[2] To date, several design strategies led to successful practical applications in living systems exploiting photo-induced electron transfer (PET),[3] internal charge transfer (ICT)[4] and fluorescence resonance energy transfer (FRET).[5] Peptides labelled with fluorescent dyes have been widely used for such purpose. However, they often involve conventional organic fluorophores such as fluorescein or rhodamine, which have a strong natural tendency to aggregate when dispersed in aqueous media due to π–π stacking interactions.[6] This aggregation generally implies a self-quenching and thus, leads to a drastic decrease of fluorescence ("turn off"), which is a thorny problem for developing efficient bioprobes. To address these issues, organic luminogens with aggregation-induced emission properties (AIE-fluorogens) have been developed.[7] These luminogens are found to be weakly or not fluorescent when molecularly dissolved but highly fluorescent when aggregated, due to the well-known restriction of the intramolecular motion (RIM) mechanism. As such, these AIE-fluorogens can be used in highly concentrated solution. They also exhibit in general higher sensitivity and better photo-bleaching resistance than conventional organic fluorophores.[8] By exploiting such strategy, highly specific AIE-fluorogens probes have been notably prepared through the incorporation of specific peptide-based recognition elements for integrin and caspase activity studies,[9] and targeting transferrin receptor.[10] Click-chemistry strategy was commonly used, allowing efficient linkage in aqueous media and without by-products, starting from azide-functionalized AIE-fluorogen and alkyne-peptide both in solution and on solid-phase,[9a, 11] as well as thiourea or amide linkages.[12] An alternative to prosthetic groups anchoring is direct incorporation of fluorescent amino acids[13] and many efforts have converged to the development of various fluorescent structures that may potentially be carried by the amino acid side chain, including phospholyl(borane) that we recently described.[14] Herein, we exploit the structural feature of silole to design new highly luminescent amino acids. Silole derivatives are a class of archetypal AIE-fluorogens with fascinating properties such as high thermal stability, high photo-stability and easy synthetic tunability.[15] Based on the previous work of our laboratory on silylated amino acids,[16] and especially, the study of the hydrosilylation reaction,[17] we incorporated 1-methyl-2,3,4,5-tetraphenyl-1H-silole into amino acids through hydrosilylation reactions with alkene- or alkyne- functionalized amino acids.[18] After selective deprotection of carboxylic function or amine, the C- or N-peptide coupling in solution and on solid phase peptide synthesis (SPPS) of the silole amino acids proved their possible incorporation into peptides. The resulting silole amino acids and peptides exhibit fluorescent properties with AIE effect.

Results and Discussion

The commercially available Boc-allylglycine **1** was first transformed into the corresponding methyl ester **2** with trimethylsilyldiazomethane in 90% yield (Scheme 1).



Scheme 1. Hydrosilylation of protected allylglycine **2**.

This fully protected allylglycine **2** was then submitted to hydrosilylation reaction with 1-methyl-2,3,4,5-tetraphenyl-1H-silole **3** in the presence of different platinum catalysts to select the most effective one (Table 1). When tetrabutylammonium hexachloroplatinate (IV) was used in refluxing dichloromethane, no reaction occurred even when doubling the quantity of silole

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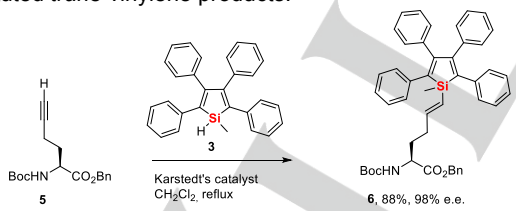
(entries 1 and 2, Table 1). Compound **4** was obtained in 72% yield with Speier's catalyst, $H_2PtCl_6.6H_2O$ (entry 3, Table 1). Interestingly, switching to Karstedt's catalyst led to further improved yield (95%) as well as shorter reaction time (only 2 h are sufficient to convert **2** into **4** instead of 8 h). The success of the hydrosilylation reaction was confirmed by IR spectroscopy since the Si-H band of the silole reagent precursor **3** at ν = 2118 cm^{-1} disappeared in the IR spectrum of **4** (Figure S1, Supporting Information). The optical purity of **4** was verified on HPLC using a chiral column by comparison with a racemic sample (See Supporting Information). Formation of silole amino acid **4** was also confirmed by ^{1}H, $^{13}C\{^{1}H\}$, $^{29}Si\{^{1}H\}$ NMR spectroscopies in $CDCl_3$ (See supporting information). Indeed, significant $^{29}Si\{^{1}H\}$ NMR chemical shift differences were observed between the silole **3** (δ = -11 ppm) and the hydrosilylation product **4** (δ = -17.7 ppm).

Table 1. Optimization of the reaction conditions of the hydrosilylation of protected allylglycine **2** with silole **3**.

Entry	Catalyst[a]	Eq. of **3**	Reaction time (h)	Yield (%)[b]
1	$(Bu_4N)_2PtCl_6$	2	12	n.d.
2	$(Bu_4N)_2PtCl_6$	4	12	n.d.
3	$H_2PtCl_6.6H_2O$	2	8	72
4	Karstedt	2	2	95

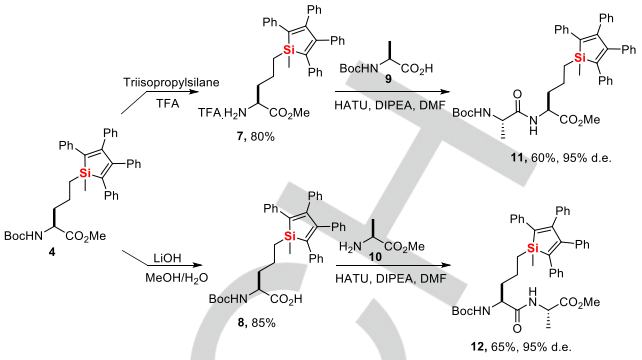
[a] 5 mol% per mol of **2**. [b] Isolated yield after purification by flash chromatography on silica gel with EtOAc/petroleum ether (1/9) as eluent.

Using these optimized conditions (Table 1, entry 4), the N-protected alkyne amino ester **5** was transformed into the corresponding silole **6** (Scheme 2). Only one signal was observed by $^{29}Si\{^{1}H\}$ NMR spectroscopy at δ = -2.1 ppm. ^{1}H NMR spectrum of **6** revealed a *trans*-product probably due to the steric hindrance of the silole moiety. Doublets assigned to the vinylic protons appear at 6.23 and 5.83 ppm with a coupling constant of ~19 Hz, a value generally observed for silane substituted *trans*-vinylene products.[19]



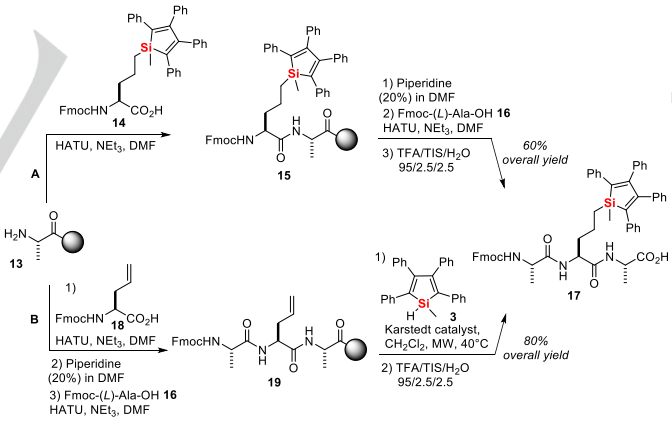
Scheme 2. Hydrosilylation of alkyne amino acid **5**.

The fully protected silole amino acid **4** was selectively N- or C-deprotected in order to prove the coupling feasibility (Scheme 3). The amine function was deprotected under acidic conditions (TFA) to afford the corresponding ammonium salt **7** in 80 % yield. The methyl ester was saponified with lithium hydroxide to afford the free carboxylic acid **8** in 85% yield. The peptide coupling of **7** and **8** was achieved under classical conditions (HATU, DIPEA, DMF) with alanine moiety as model (**9** and **10**).



Scheme 3. Synthesis of silole dipeptide derivatives.

Solid phase peptide synthesis (SPPS) was demonstrated by synthesizing the tripeptide **17** with the N-Fmoc protected derivative **14** (Scheme 4, pathway A). The coupling step between Ala-Wang **13** and silole **14** was first carried out using HATU and Et_3N in DMF. Then, dipeptide **15** was deprotected with 20 % piperidine solution in DMF and coupled with Fmoc-(*L*)-Ala-OH **16.** Final cleavage with TFA/TIS/H_2O afforded the corresponding tripeptide **17** with a silole group in 60 % overall yield. On the other hand, tripeptide **19** obtained by SPPS from Ala-Wang **13** in 88 % overall yield was subjected to hydrosilylation with silole **3**, directly on support under microwaves irradiations in CH_2Cl_2 at 40°C for 2 hours. Silole-containing tripeptide **17** was finally obtained in higher yield of 80 % after final cleavage by this strategy.



Scheme 4. Solid Phase Peptide Synthesis (SPPS).

The optical properties of the new silole amino acids and peptides were then studied. The UV-visible absorption and photoluminescence (PL) spectra of these silole derivatives were recorded in THF at a concentration of 5.10^{-4} M (Figure 1 and S11-S22, Supporting Information). All the silole amino acids and peptides exhibit an absorption band (λ_{abs}) between 350 and 365 nm ascribed to the π–π^* transition of the silacyclopentadiene ring (Table 2).[20]

Table 2. Absorption and emission characteristics of silole amino acids and peptides at room temperature.

Entry	Compound	λ_{abs} (nm)[a]	λ_{ex} (nm) [a]	λ_{em} (nm) [a]	$\Delta\lambda$	ϕ_{THF} (%) [a]	ϕ_{agg} (%) [a]	I_{agg}/I_{THF}
1	**4**	287 (sh)[b], 358	360	491	131	1	5	5.6
2	**6**	290 (sh), 365	380	500	120	2	12	44
3	**11**	290, 354	380	495	115	1	10	5.8
4	**12**	286, 350	380	492	112	1	8	7.3
5	**17**	289 (sh), 365	400	502	102	1	8	6.4

[a] measured in THF or THF/H_2O at 25°C. [b] sh = shoulder.

Silole amino acids and peptides **4**, **6**, **11**, **12** and **17** are weakly emissive in THF, usually a good solvent, with emission maxima between 485 and 500 nm and large Stokes shifts (~100-130 nm).

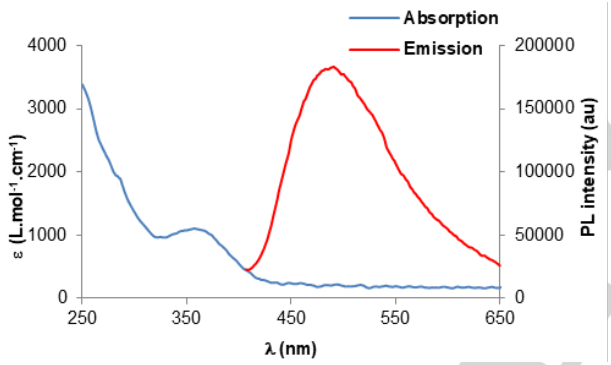
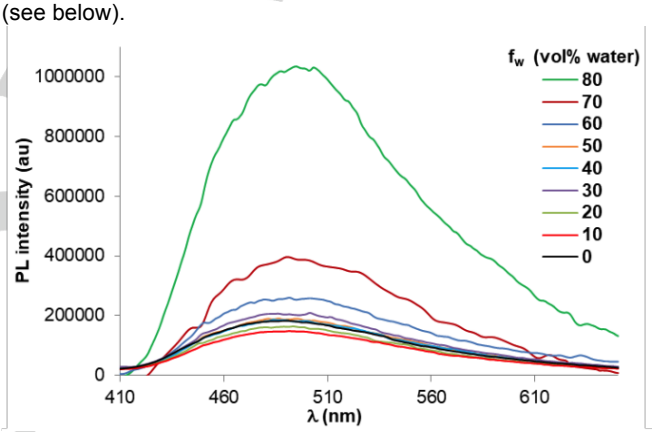


Figure 1. UV-Vis absorption and emission spectra of **4** in THF (C: 5.10^{-4} M, λ_{exc} = 360 nm).

The AIE properties of silole amino acids and peptides were then studied in THF/water mixtures with different water fractions (f_w) in view of fine-tuning the water content as well as the aggregation extent (Figure 2). Each PL intensity was divided by its maximum PL intensity (i.e. the PL intensity for the highest f_w) to get I/I_0 ranging from 0 to 1. As expected, the greater the water fraction, the more the fluorescence increases; molecular aggregation inducing a slight red shift (~5-10 nm) in the emission spectrum. As shown in Figure 2 and Figure S15-S18 in the Supporting Information, when f_w varies from 0 to 60 % vol, the fluorescence remains very low. A remarkable increase in the fluorescence was noted after the water fraction reached 70 and 80 % vol, typical from AIE-fluorogens leading to I_{agg}/I_{THF} ~ 6 (except for **6**, I_{agg}/I_{THF} ~ 44).

These silole amino acids and peptides differ by the nature of the substituents introduced on the silicon atom. However, the effect on absorption and emission maxima of the substituents on the 1,1 position is known to be rather small and mostly due to inductive effects.[21] As such, the trend observed in the absorption and emission maxima cannot be related to the difference in the inductive effects between the substituents due to their similarity but rather on value of the torsion angles between the central silole ring and the adjacent phenyl rings (see below).



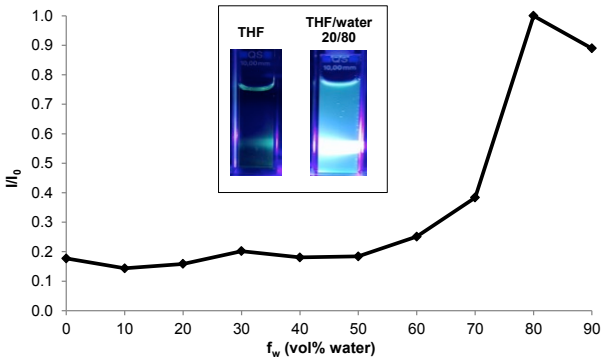


Figure 2. Emission spectra of **4** in THF/water mixtures with different water fractions (f_w) (Concentration: 5.10^{-4} M, λ_{exc} = 360 nm) (top). Plot of I/I_0 values *vs.* water fractions. I_0 is the PL intensity for the highest f_w (bottom). Inset: photos of silole amino acid **4** under the illumination of a UV lamp at 405 nm in THF\water mixture (f_w = 0 and 80%).

To gain insight into the relationship between the optical properties and the conjugation pattern in silole amino acids **4** and **6**, density functional theory calculations were performed. B3LYP method with 6-31G* basis set was used for the

calculation.[22] The optimized structures and the orbital distributions of the HOMO and LUMO energy levels of silole amino acids **4** and **6** are depicted in Figure 3. As previously observed, the four phenyl rings decorating the central silacyclopentadiene ring adopt a propeller-like arrangement (Figure S23, Supporting Information).[23]

The selected parameters for these compounds are summarized in Table S1 (Supporting Information). Interestingly, the average value of the torsion angles between the central silole ring and the adjacent phenyl rings at the 2,5-positions decreases going from 51.2° for **4** to 46.8° for **6**, suggesting a more efficient π-conjugation over the three benzene-silole-benzene rings. This difference originates from the disposition of the substituents at the 1,1-position. Indeed, the double bond in **6** allows the CH_3-SiC_2-CH=CH- fragment adopting an eclipsed conformation whereas a gauche conformation of the CH_3-SiC_2-CH_2-CH_2- fragment is found in **4**, leading to an increase of the torsion angle of the adjacent phenyl ring. The DFT calculations at the B3LYP/6-31G* level on amino acids **4** and **6** were carried out to gain more insight into their electronic structures (Figure 3). The orbital plots **4** and **6** are typical for the HOMO and LUMO orbitals of siloles, *i.e.* the LUMO of the silole ring displays a typical σ(Si–$C_{exocyclic}$)*–π(butadiene)* hyperconjugation responsible for its remarkable electronic properties.[15a, 21] With the exception of carbon atoms directly connected to the silicon atom, a negligible electronic density was noted on the chains bearing the amino acid moiety. The decrease of 70 meV in the energy band gap from **4** to **6** is in good agreement with the red-shifted absorption and emission spectra of **6** compared to **4**.

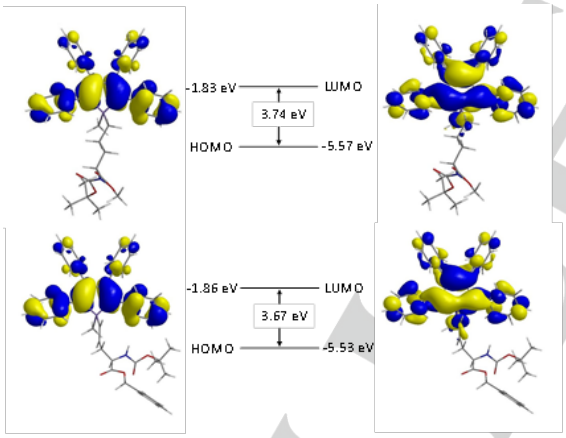


Figure 3. DFT-B3LYP/6-31G* calculated molecular orbital amplitude plots of the HOMO and LUMO energy levels of silole amino acids **4** (top) and **6** (bottom).

Conclusions

In conclusion, new silole amino acids were synthesized by hydrosilylation of alkyne or alkene amino acids. After selective deprotection of carboxylic function or amine, C- or N-peptide coupling in solution and on solid phase proved their possible incorporation into peptides. The silole moiety can be also grafted on a precursor peptide directly on the solid support. Such silole amino acids and peptides exhibit AIE properties with λ_{em} around 500 nm and $\Delta\lambda$ ~ 100 nm. As such, this approach constitutes an alternative and promising strategy for incorporation of AIE fluorogens in peptides.

Experimental Section

General remarks: All reactions involving air-sensitive reagents were performed under argon. Solvents and reagents were purchased from commercial sources and used without purification. THF was freshly distilled from benzophenone/sodium prior to use. All reagents were purchased from commercial sources and used without purification. 1-methyl-2,3,4,5-tetraphenyl-1H-silole (**3**) was prepared according to the literature method.[24] Thin layer chromatography was carried out using silicagel 60 F254 sheets. Spots were visualized by treatment with an ethanolic solution of ninhydrin 10%, followed by heating. Purifications were performed with column chromatography using silica gel 60 (230-400 mesh). Melting points were determined on a Buchi 510 melting point apparatus and are uncorrected. Nuclear magnetic resonance ^{1}H, $^{13}C\{^{1}H\}$ and $^{29}Si\{^{1}H\}$ NMR spectra were recorded on 300, 400 or 500 spectrometer at room temperature. Data are reported as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. s = broad signal, coupling constant(s) in Hertz, integration. Low resolution electrospray ionization (ESI) mass spectra were recorded on a Micromass Platform Electrospray mass spectrometer. Spectra were recorded in the positive mode (ESI^+). UV chromatogram was performed using an Agilent 1220 HPLC equipped with a Chromolith® high resolution C-18 column (50 µm x 4.6 mm). High resolution mass spectra (HRMS) were recorded using a Synapt G2-S mass detector operating at capillary tension of 1 kV and cone tension of 20 V. Optical rotations values were measured on a Perkin-Elmer 341 polarimeter (20°C, 589 nm). Chiral HPLC analyses were performed on a Beckman chromatograph with a variable detector at λ = 210 nm and λ = 254 nm using a chiral column OD-H, AS-H or AD-H (0.46 x 25 cm). Ultra-violet absorption spectra were recorded at 25°C on a JASCO V-750 spectrophotometer in 10 mm quartz cells (Hellma); abbreviation used sh, shoulder. All the extinction coefficients were determined by preparing solutions of silole amino-acids at different concentrations (at least five different concentrations) through the Beer-Lambert relationship. The concentration solution range was judiciously chosen to stay in the linear range of the Beer-Lambert relationship (A ~ 0.2-0.8). Emission spectra were recorded at 25°C on a fluorescence spectrometer (FS920, Edinburgh Instruments) equipped with a calibrated photomultiplier in a Peltier (air cooled) housing (R928P, Hamamatsu), with a 450 W continuous xenon arc lamp as the excitation source for steady-state photoluminescence measurements using a quartz cuvette with 1.0 cm excitation path length. Emission and excitation spectra were corrected for the wavelength response of the system using correction factors supplied by the manufacturer. The quantum yields of the samples were determined using an integrating sphere (120 mm diameter) of Edinburgh Instruments under air.

Methyl (S)-2-((*tert*-butoxycarbonyl)amino)pent-4-enoate 2: To a solution of Boc-allylglycine **1** (500 mg, 2.32 mmol) in toluene/MeOH (2/3, 20 mL) under argon at 0°C was added dropwise $TMSCHN_2$ (1.74 mL, 3.48 mmol, 2M in hexane). The mixture was stirred at r.t. during 1 hour then quenched with 1 mL of acetic acid and evaporated to dryness. The crude product was purified by flash column chromatography on silica gel with petroleum ether/ethyl acetate (80:20) to afford desired compound **2** with 90 % yield. The spectroscopic data are in accordance with the literature.[25]

General procedure for hydrosilylation reaction: To a solution of alkene or alkyne (1 eq.) in anhydrous CH_2Cl_2 (7.7 mL / mmol of amino acid) under argon were added 1-methyl-2,3,4,5-tetraphenyl-1H-silole **3** (2

eq.) and catalyst (5 mol %). The mixture was stirred at reflux and monitored by TLC (petroleum ether/ethyl acetate 90:10) until reaction was completed. The solvent was removed and the crude product was purified by flash column chromatography on silica gel with petroleum ether/ethyl acetate (90:10) to afford the corresponding 1-methyl-2,3,4,5-tetraphenylsilole amino esters **4** and **6**.

Methyl (S)-2-((*tert*-butoxycarbonyl)amino)-5-(1-methyl-2,3,4,5-tetraphenyl-1H-silol-1-yl)pentanoate 4: Following the general procedure, Boc-AllylGly-OMe 1 (100 mg, 0.43 mmol), **3** (344 mg, 0.86 mmol) and Karstedt catalyst (10 μL, 0.02 mmol) were used to afford the silole amino ester **4** in 95 % yield as a yellow sticky solid. R_f = 0.48 (petroleum ether/ethyl acetate 8:2). $[\alpha]_D$ = -31 (c = 1, $CHCl_3$). Enantiomeric excess > 99 % was determined using chiralcel AD-H, *n*-hexane/*i*PrOH (98:2), 0.8 mL.min^{-1}, λ = 214 nm, 30°C, t_R (*S*) = 9.7 min, t_R (*R*) = 11.4 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (ddd, $J_{H,H}$ = 6.1, 5.4, 2.4 Hz, 5H, Harom), 7.02 – 6.99 (m, 5H, Harom), 6.92 – 6.88 (m, 5H, Harom), 6.82 – 6.78 (m, 5H, Harom), 4.82 (d, $^3J_{H,H}$ = 8.2 Hz, 1H, NH), 4.23 (q, $J_{H,H}$ = 13.4, 7.7 Hz, 1H, CHN), 3.64 (s, 3H, O-CH_3), 1.84 – 1.73 (m, 1H, CH-Cα), 1.65 – 1.55 (m, 2H, CH_2), 1.47 – 1.43 (m, 1H, CH-Cα), 1.42 (s, 9H, $(CH_3)_3$), 1.09 – 0.98 (m, 1H, CH-Si), 0.94 – 0.85 (m, 1H, CH-Si), 0.47 (s, 3H, CH_3-Si). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 173.5, 155.5, 155.1, 155, 140.9, 140.8, 140, 139.9, 138.8, 130.1, 128.9, 128.1, 127.56, 126.4, 125.7, 79.9, 53.3, 52.2, 35.9, 28.5, 19.9, 13.1, -5.3. ^{29}Si{^{1}H} NMR (99 MHz, $CDCl_3$) δ -17.7 (s). HRMS calculated for $C_{40}H_{44}NO_4Si^+$ $[M+H]^+$ 630.3034 found 630.3031. UV-Vis (THF): λ (ε L.mol^{-1}.cm^{-1}) = 287 (1860), 358 (1080) nm.

Benzyl (*S*),(*E*)-2-((*tert*-butoxycarbonyl)amino)-6-(1-methyl-2,3,4,5-tetraphenyl-1H-silol-1-yl)hex-5-enoate 6: Following the general procedure, alkyne **5** (100 mg, 0.31 mmol), **3** (252 mg, 0.62 mmol) and Karstedt catalyst (8 μL, 0.02 mmol) were used to afford the silole amino ester **6** in 88 % yield as a yellow sticky solid. R_f = 0.52 (petroleum ether/ethyl acetate 8:2). $[\alpha]_D$ = -28 (c = 1, $CHCl_3$). Enantiomeric excess > 99 % was determined using chiralcel AD-H, *n*-hexane/*i*PrOH (99:1), 0.8 mL.min^{-1}, λ = 214 nm, 30°C, t_R (*S*) = 17.5 min, t_R (*R*) = 24.5 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 5H, Harom), 7.04-7.01 (m, 10H, Harom), 6.94-6.88 (m, 5H, Harom), 6.83-6.77 (m, 5H, Harom), 6.23 (dt, $J_{H,H}$ = 18.6, 6.1 Hz, 1H, -CH=), 5.83 (d, $J_{H,H}$ = 18.6 Hz, 1H, =CH-Si), 5.23-5.12 (m, 2H, O-CH_2), 5.00 (d, $J_{H,H}$ = 7.7 Hz, 1H, NH), 4.37-4.28 (m, 1H, CHN), 2.21-2.15 (m, 1H, CH-Cα), 1.74-1.67 (m, 1H, CH-Cα), 1.44 (s, 9H, $(CH_3)_3$), 0.94-0.88 (m, 2H, CH_2-C=), 0.54 (s, 3H, CH_3-Si). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.5, 154.8, 140.3, 139.6, 138.9, 138.6, 135.4, 129.9, 129.8, 129, 128.9, 128.6, 128, 127.9, 127.5, 79.8, 67, 53.2, 31.5, 28.3, -6.0. ^{29}Si{^{1}H} NMR (99 MHz, $CDCl_3$) δ -2.1 (s). HRMS calculated for $C_{47}H_{48}NO_4Si^+$ $[M+H]^+$ 718.3347 found 718.3346. UV-Vis (THF): λ (ε L.mol^{-1}.cm^{-1}) = 290 (157100), 365 (14130) nm.

Methyl-(S)-2-amino-5-(1-methyl-2,3,4,5-tetraphenyl-1H-silol-1-l)pentanoate 7: Silole amino ester **4** (50 mg, 0.08 mmol) was dissolved in 0.6 mL of HCl (4M in dioxane). The mixture was stirred at room temperature for 2 hours and the solvent evaporated under *vacuum*. The residue was triturated in Et_2O to afford the desired compound **7** in 95 % yield. The crude product was used without further purification for peptide coupling. LC-MS: t_R = 2.36 min. ESI-MS: 530.3 $[M+H]^+$, 548.2 $[M+H_3O]^+$ (λ = 214 nm).

(S)-2-((*tert*-butoxycarbonyl)amino)-5-(1-methyl-2,3,4,5-tetraphenyl-1H-silol-1-yl)pentanoic acid 8: To a solution of silole amino ester **4** (30 mg, 0.05 mmol) in THF/MeOH (1:1, 500 μL) was added LiOH (4 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 2 hours and the solvent was removed under *vacuum*. The residue was taken up in water and extracted with Et_2O. The aqueous layer was then acidified to pH 3 to 4 with $KHSO_4$ (1M) and extracted with EtOAc (3 x 5 mL). The organic layer was dried over magnesium sulfate, filtered and evaporated to dryness to afford the amino acid **8** as a yellow sticky solid with 80 % yield. The amino acid **8** was used without further purification for peptide coupling. LCMS: t_R = 2.57 min. ESI-MS: 613.3 $[M+H]^+$, 638.2 $[M+Na]^+$ (λ = 214 nm).

Dipeptide 11: To a solution of Boc-Ala-OH (14 mg, 0.076 mmol) in DMF (800 μL) were added HATU (31 mg, 0.083 mmol) and DIPEA (47 μL, 0.29 mmol). After 10 minutes, silole amino ester **7** (43 mg, 0.076 mmol) was added and the mixture was stirred overnight at room temperature. DMF was evaporated and the residue was taken up in EtOAc and washed with aqueous saturated $NaHCO_3$, $KHSO_4$ (1M) and brine. The organic layer was dried on magnesium sulfate, filtered and evaporated to afford the dipeptide **11** with 65 % yield as yellow sticky solid. R_f = 0.22 (petroleum ether/ethyl acetate 7:3). Enantiomeric excess > 99 % was determined using chiralcel AD-H, *n*-hexane/iPrOH (95:5), 0.8 mL.min^{-1}, λ = 214 nm, 30°C, t_R (*S,R*) = 28.7 min, t_R (*S,S*) = 32.3 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13-6.78 (m, 20H, Harom), 6.45 (d, $^3J_{H,H}$ = 8.0 Hz, 1H, NH amide), 4.88 (d, $^3J_{H,H}$ = 10.3 Hz, 1H, NHBoc), 4.42 (td, $J_{H,H}$ = 7.9, 5.3 Hz, 1H, CHN), 4.04 (dt, $J_{H,H}$ = 11.7, 5.9 Hz, 1H, CHN), 3.59 (s, 3H, O-CH_3), 3.45-3.17 (m, 1H, CH_2), 1.36 (d, $^3J_{H,H}$ = 5.1 Hz, 9H, $(CH_3)_3$), 1.22 (dd, $J_{H,H}$ = 6.8, 2.0 Hz, 3H, CH_3), 1.04 (t, $J_{H,H}$ = 7.1 Hz, 2H, CH_2), 0.88-0.73 (m, 2H, CH_2-Si), 0.27 (d, $J_{H,H}$ = 6.6 Hz, 3H, CH_3-Si). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.4, 156.9, 156.8, 144.9, 143.2, 142.5, 140, 137.7, 136.2, 136.2, 130, 129.7, 129.6, 129.6, 129.6, 129.3, 128.3, 128.3, 128.2, 127.7, 127.5, 127.4, 127.4, 127, 126.2, 125.5, 52.3, 51.8, 46.2, 41.8, 40.3, 35.6, 29.7, 28.4, 28.3, 19.7, 19.3, 17.4, 17.2, 14.6, 12.9. ^{29}Si{^{1}H} NMR (99 MHz, $CDCl_3$) δ -15.8 (s). FTIR (neat): $\tilde{\nu}$ = 3042, 1582, 1505, 1437, 1326, 1211, 1028, 810, 747, 643 cm^{-1}. HRMS calculated for $C_{43}H_{48}N_2O_5Si$ $[M+H]^+$ 701.3405 found 701.3404. UV-Vis (THF): λ (ε L.mol^{-1}.cm^{-1}) = 290 (23970), 354 (18780) nm.

Dipeptide 12. To a solution of silole amino acid **8** (23 mg, 0.037 mmol) in DMF (400 μL) were added HATU (0.041 mmol, 16 mg) and DIPEA (0.13 mmol, 21 μL). After 10 minutes, HCl.H-Ala-OMe (0.041 mmol, 5.7 mg) was added and the mixture was stirred overnight at room temperature. The DMF was evaporated and the residue taken up in EtOAc and washed with aqueous saturated $NaHCO_3$, $KHSO_4$ (1M) and brine. The organic layer was dried on magnesium sulfate, filtered and evaporated to afford the dipeptide **12** with 60 % yield as a yellow sticky solid. R_f = 0.24 (petroleum ether/ethyl acetate 4:1). Enantiomeric excess > 99 % was determined using chiralcel AD-H, hexane/*i*PrOH (95:5), 0.8 mL.min^{-1}, λ = 214 nm, 30°C, t_R (*S,R*) = 28.1 min, t_R (*S,S*) = 31.5 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15-6.77 (m, 20H, Harom), 6.52 (d, $^3J_{H,H}$ = 7.2 Hz, 1H, NHamide), 4.74 (br. s, 1H, NHBoc), 4.61-4.48 (m, 2H, 2 x CHN), 3.72 (s, 3H, OCH_3), 3.43-3.33 (m, 2H, CH_2), 1.43 (d, $^3J_{H,H}$ = 5.2 Hz, 9H, $(CH_3)_3$), 1.26 (d, $^3J_{H,H}$ = 7.1 Hz, 3H, CH_3), 0.97-0.83 (m, 2H, CH_2-CH_2-Si), 0.48 (s, 3H, CH_3-Si). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 172.5, 155.2, 142.3, 142.1, 140.4, 139.9, 137.8, 137.7, 135.7, 135.6, 134.4, 128.4, 128.2, 128.1, 128.0, 127.9, 127.7, 126.3, 126.2, 125.9, 125.8, 125.4, 125.1, 124.4,124.3, 123.7, 38.90, 27.7, 21.9, 12.5. ^{29}Si{^{1}H} NMR (99 MHz, $CDCl_3$) δ -15.8 (s). FTIR (neat): $\tilde{\nu}$ = 3000, 1582, 1506, 1436, 1334, 1211, 1025, 812, 748, 644 cm^{-1}. HRMS calculated for $C_{43}H_{48}N_2O_5Si$ $[M+H]^+$ 701.3405 found 701.3403. UV-Vis (THF): λ (ε L.mol^{-1}.cm^{-1}) = 286 (23440) 354 (19900) nm.

(*S*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-5-(1-methyl-2,3,4,5-tetraphenyl-1H-silol-1-yl)pentanoic acid 14: To a solution of silole amino acid hydrochloride (100 mg, 0.19 mmol) in 1.5 mL of aqueous sodium carbonate (10 %), was added Fmoc-Cl (88 mg, 0.38 mmol) in dioxane (1.5 mL) at 0°C. After 2 hours stirring, the reaction mixture was diluted with H_2O (3 mL) and extracted with Et_2O (2 x 3 mL). The aqueous layer was acidified with HCl (1N) to pH 2 and extracted with AcOEt (3 x 3 mL). The combined organic layers were dried over $MgSO_4$, and evaporated under *vacuo* to afford the corresponding *N*-Fmoc silole amino acid **14** as a yellow sticky solid in 60 % yield. ^{1}H NMR (400 MHz,

$CDCl_3$) δ 7.49-7.44 (m, 4H, H_{Fmoc}), 7.30-7.25 (m, 4H, H_{Fmoc}), 7.16-7.01 (m, 15H, H_{arom}), 6.78 (dd, $J_{H,H}$ = 8.2, 1.3 Hz, 5H, Harom).4.55-4.42 (m, 2H, O-CH_2), 4.30 (dd, $J_{H,H}$ = 48.4, 7.8 Hz, 1H, CH_{Fmoc}), 3.73-3.57 (m, 1H, CHN), 1.64-1.44 (m, 2H, CH_2), 1.16-0.98 (m, 2H, CH_2), 0.26 (dd, $J_{H,H}$ = 15.0, 7.9 Hz, 2H, CH_2), -0.12 (s, 3H, CH_3, Si).$^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 174.5, 156.1, 154.9, 140.1, 139.4, 138.7, 138.5, 136.1, 135.8, 133.6, 130.0, 129.7, 129.4, 128.7, 128.5, 128.1, 127.9, 127.7, 127.4, 79.7, 67.1, 53.7, 31.4, 28.1, -5.4. $^{29}Si\{^1H\}$ NMR (99 MHz, $CDCl_3$) δ -16.5 (s). FTIR (neat): $\tilde{\nu}$ = 2988, 2912, 1656, 1593, 1484, 1442, 1248, 1018, 765, 700 cm^{-1}. HRMS calculated for $C_{49}H_{44}NO_4Si$ $[M+H]^+$ 738.3034 found 738.3031.

Procedure for preparation of tripeptide 17

Pathway A: A solution of N-Fmoc-protected silole amino acid **14** (30 mg, 40 µmol), HATU (16 mg, 44 µmol) and NEt_3 (16 µL, 120 µmol) in DMF (0.6 mL) was added to H-Ala-Wang resin **13** (40 mg, 1 mmol/g). The mixture was stirred in a 5 mL syringe for 10 min, then washed with (2 x 3 mL) of DMF. Then the dipeptide **15** was deprotected with piperidine 20% in DMF (3 x 1 mL) and washed with 3 mL of DMF. A solution of (*L*)-Fmoc-Ala-OH (56 mg, 160 µmol), HATU (66 mg, 176 µmol) and NEt_3 (67 µL, 480 µmol) in 0.6 mL of DMF was added to the previous deprotected dipeptide. After 10 min stirring, the resin was washed with DMF (3 mL), CH_2Cl_2 (3 mL) and the final cleavage from the resin was performed with 1 mL of TFA/TIS/H_2O (95/2.5/2.5) for 30 min. The solution was concentrated under *vacuo* and the residue was washed with Et_2O (2 x 3 mL) to afford the tripeptide **17** in 60% overall yield.

Pathway B: A solution of N-Fmoc-Allylglycine **18** (134 mg, 0.40 mmol), HATU (152 mg, 0.4 mmol) and NEt_3 (70 µL, 0.6 mmol) in DMF (2 mL) was added to H-Ala-Wang resin **13** (100 mg, 1 mmol/g). The mixture was stirred in a 5mL syringe for 30 min, then washed with (2 x 3 mL) of DMF. Then the dipeptide was deprotected with piperidine 20% in DMF (3 x 1 mL) and washed with 3 mL of DMF. A solution of (*L*)-Fmoc-Ala-OH (124 mg, 0.4 mmol), HATU (152 mg, 0.44 mmol) and NEt_3 (70 µL, 0.6 mmol) in 2 mL of DMF was added to the previous deprotected dipeptide. After washing with DMF and CH_2Cl_2, the resin was transferred into a sealed vial with tetraphenylsilole **3** (80 mg, 0.2 mmol), Karstedt's catalyst (10 µL, 0.02 mmol) and dichloromethane (1.5 mL). The mixture was heated under microwaves at 40°C for two hours. The final cleavage from the resin was performed with 1 mL of TFA/TIS/H_2O (95/2.5/2.5) for 30 min. The solution was concentrated under *vacuo* and the residue was washed with Et_2O (2 x 3 mL) to afford the tripeptide **17** in 80 % overall yield. $^{29}Si\{^1H\}$ NMR (99 MHz, $CDCl_3$) δ –12.3 (s). LCMS: t_R = 1.52 min, $[M+H]^+$ 880.1, $[M+H_3O^+]$ 898.4. HRMS for $C_{55}H_{53}N_3O_6Si$ $[M+H]^+$ calculated 880.3776 found 880.3774. UV-Vis (THF): λ (ε L mol^{-1} cm^{-1}) = 289 (17100), 365 (15860) nm.

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Keywords: Silole • Amino acids • Hydrosilylation • Fluorescent probe • Aggregation-Induced Emission

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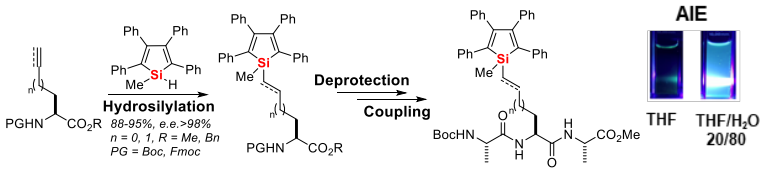

Silole amino acids were obtained by hydrosilylation of unsaturated amino acids with yield up to 95%. After selective deprotection, C- or N-peptide coupling was performed in solution or on solid support. They exhibit AIE properties (λem ~500 nm; Δλ ~100 nm) and constitute a promising strategy for incorporation of AIE fluorogens in peptides.

AIEgens Silole amino acids *

M. Arribat, E. Rémond, S. Richeter, P. Gerbier; S. Clément, F. Cavelier**

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Silole amino acids with Aggregation-Induced Emission features synthezised by hydrosilylation reaction