Synthesis of new fluorescent pyrylium dyes and study of their interaction with N-protected amino acids

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Abstract

Six new 6-styryl-2,4-diarylpyrylium salts have been synthesized and fully characterized by means of ^{1}H / ^{13}C NMR, HRMS, UV-vis and Steady-State / Time-Resolved Fluorescence spectroscopies. This set of molecules is comprised by a core pyrylium fluorophore an amino acid (valine or phenylalanine) and an alkylic chain of variable length. The emissive properties (fluorescence quantum yields and lifetimes) in dichloromethane, acetonitrile and PBS have been recorded. The interaction of these pyrylium salts with aminoacids in their N-protected forms has been studied by means of fluorescence quenching, using the Stern-Volmer methodology. It has been found that dynamic (collisional) quenching is the most prevalent process for all the fluorescent molecules, irrespective of the amino acid building block or the length of the alkyl chain. The emission of the pyrylium molecules is strongly quenched by Z-Trp-OH and to a lesser extent by Z-Tyr-OH and Z-Met-OH and no quenching was measured with Z-Ala-OH, Z-Val-OH and Z-Phe-OH. A small degree of ground-state complexation was observed for receptor **8a** and by Z-Trp-OH (upward curvature in the Stern-Volmer plot). Complementary ^{1}H-NMR titrations demonstrated the existence of such weak ground state complex.

Introduction

The association of molecular recognition principles with the presence of an optical signal has led to the emergence of the area coined by E. V. Anslyn 'Supramolecular Analytical Chemistry'.[1,2] Rapid expansion of the field in recent years has led to a huge variety of chemical synthetic receptors with an optical readout, frequently fluorescence emission. Hence, analytical techniques using fluorescence are particularly important in the development of supramolecular chemosensors with practical applications in fields such as imaging of biomolecules for diagnostics, or environmental monitoring for detection of pollutants, to mention only two examples. Sensitivity of fluorescence is unsurpassed by other traditional analytical tools and this is one of the reasons for the massive use of this technique in recent years. [3–11] Changes in the fluorescence intensity or in the fluorescence lifetimes report changes in the environment of the fluorophore that can be associated to the recognition event. Simple methodologies like the Stern-Volmer analysis have contributed to elucidate various processes, from the mechanisms involved in pollutant degradation[13] and photo-redox catalysis[14] to the complexities of amino acid recognition.[11,15–17] In this last regard, recently it has been proved that amino acids with electron-rich side chains, such as tryptophan, cause fluorescence quenching on dyes widely used in Molecular Biology, which can be relevant in biological studies.[18–20]

A large variety of molecular architectures has been used so far for the construction of chemosensing systems showing fluorescence features. The group of M. Levine has recently reviewed the field and has collected the fluorophores typically used for the synthesis of supramolecular sensors: bodipys, squaraines, cyanines, xanthenes, acridines, isoquinolines, coumarins, perylene diimides, etc.[21] Pyrylium dyes are not commonly used to construct supramolecular receptors, despite this class of dyes have particular easy synthesis and straightforward purification. This fact is in great contrast with the increasing use of pyrylium salts in other areas of research, like, for instance photocatalysis,[22–26] dye sensitized solar cells,[27] liquid crystals,[28] carbon nanotube chemistry,[29] and photodynamic therapy.[30] In the analytical context molecules with a pyrylium fluorophore have been employed as chemodosimeters for nitric oxide sensing,[31] semiconductor quantum dots characterization,[32] polarity probes,[33] protein

analysis,[34] determination of metabolites in food[35] and quantification of anionic species like cyanide[36] and sulfide.[37] From the physical chemistry viewpoint, pyrylium salts have also attracted recently the attention since they can show important cation-pi interactions.[38,39]

Herein, we present the synthesis and chemical and photophysical characterization of six new fluorescent pyrylium salts, carrying an aminoacid moiety and a chain of different length in the structure, Also the supramolecular studies (Stern-Volmer analysis) on the interaction with a series of N-protected amino acids are presented. As it will be shown, all the pyryilum salts synthesized display absorptions in 400-500 nm, single emission bands in 500-650 nm, and N-protected amino acids (Z-Met, Z-Tyr and Z-Trp) induce mainly dynamic (collisional) quenching of the fluorescence. No quenching was measured with Z-Ala-OH, Z-Val-OH and Z-Phe-OH. It is worth to mention that collisional quenching could be of practical utility, as exemplified by the indicators for chloride developed by the group of A. S. Verkman,[40] employed to understand cystic fibrosis.[41]

Provided the lack of examples of pyrylium salts in Supramolecular Analytical Chemistry, this contribution could open the door to the synthesis of other receptors based on the pyrylium fluorophore.

Experimental section

Materials and instruments

All commercially available reagents and solvents were used as received. Deionized water was produced by a Milli-Q water purification system. Compound **1c** was purchased from Aldrich. Fourier Transform Infrared (FT-IR) spectra were acquired using a FT-IR-6200 type A JASCO spectrometer, with 4 cm^{-1} resolution and 50 scans accumulation. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 MHz spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C). A Q-TOF Premier mass spectrometer with an electrospray source (Waters, Manchester, UK) has been used. UV–Vis absorption spectra were recorded in a Hewlett–Packard 8453 apparatus. Steady-state fluorescence spectra were recorded in a Spex Fluorog 3-11 equipped with a 450 W

xenon lamp. Time-resolved fluorescence experiments were performed using an IBH-5000U apparatus using a 464 nm (fwhm 1.4 ns) nanoLED as excitation source.

Synthetic procedure and chemical characterization.

General procedure for the synthesis of compounds 1a-c and 2a-c. 10 g of N-protected valine or phenylalanine were weighted into a 250 mL round bottom flask and were dissolved in 80 mL of THF in an ice bath with stirring under a N_2 atmosphere. 1.1 equivalents of Et_3N were added and stirred for 5 minutes. Then, 1 equivalent of ClCOOEt was added dropwise from an addition funnel. The obtained reaction mixture was stirred for 30 minutes in an ice bath (temperature 5-10 °C). After this time, 1 equivalent of the corresponding amine was added dropwise and stirred during 1 hour at 0°C. The reaction mixture was further stirred at room temperature overnight. After that, the resulting mixture was filtered, the filtrate was collected, and the solvent was vacuum evaporated. The resulting solid was dissolved in chloroform and washed with distilled water, with a saturated solution of $NaHCO_3$ and finally with brine. The organic phases were collected and dried with Na_2SO_4. The solvent was vacuum evaporated, and the resulting white solid was dried under vacuum.

Compound 1a. IR(ATR)(cm^{-1}) 3300, 2966, 1684, 1641, 1529, 1455, 1349, 1294, 1238, 1040; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.24 (m, 5H), 5.86 (s, 1H), 5.30 (s, 1H), 5.03 (d, *J* = 13.2 Hz, 2H), 3.83 (dd, *J* = 8.8, 6.4 Hz, 1H), 3.12 (m, 2H), 2.04 (m, 1H), 1.43 (dd, *J* = 14.2, 7.1 Hz, 2H), 1.01 – 0.65 (m, 8H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 171.3, 156.5, 136.3, 128.5, 128.1, 127.9, 66.8, 60.7, 41.2, 31.1, 22.7, 19.2, 18.0, 11.3; HRMS (ESI-TOF)$^+$ calculated for $C_{16}H_{24}N_2O_3^+$ $(M+H)^+$(m/z): 293.1865; experimental $(M+H)^+$ (m/z): 293.1862; $[\alpha]_D$= -13.06 (25 °C, c= 0.0127 g/mL in $CHCl_3$).

Compound 1b. IR(ATR)(cm^{-1}) 3288, 2923, 2854, 1684, 1641, 1535, 1461, 1349, 1294, 1244, 1040; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.30 (m, 5H), 6.53 (s, 1H), 5.75 (d, *J* = 9.0 Hz, 1H), 5.07 (m, 12.1 Hz, 2H), 3.99 (m, 1H), 3.27 (dd, *J* = 12.8, 6.2 Hz, 1H), 3.14 (td, *J* = 12.8, 6.9 Hz, 1H), 2.07 (dd, *J* = 12.7, 6.2 Hz, 1H), 1.45 (m, 2H), 1.25 (d, *J* = 6.0 Hz, 10H), 1.05 – 0.73 (m, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 171.3, 156.5, 136.3, 128.5, 128.1, 127.8, 66.9, 60.6, 39.5, 31.8, 31.1, 29.5, 29.2, 29.2, 26.9, 22.6, 19.2, 18.1, 14.0.; HRMS (ESI-TOF)$^+$ calculated for $C_{21}H_{34}N_2O_3^+$ $(M+H)^+$ (m/z): 363.2648; experimental $(M+H)^+$ (m/z): 363.2648; $[\alpha]_D$= -9.91 (25 °C, c= 0.0113 g/mL in $CHCl_3$).

Compound 1c. IR(ATR)(cm^{-1}) 3294, 2916, 2854, 1690, 1646, 1535, 1467, 1349, 1294, 1244, 1040; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.30 (m, 5H), 6.29 (s, 1H), 5.60 (d, *J* = 7.7 Hz, 1H), 5.08 (m, 12.0 Hz, 2H), 3.96 (m, 1H), 3.27 (s, 1H), 3.17 (dt, *J* = 12.9, 6.3 Hz, 1H), 2.09 (d, *J* = 5.8 Hz, 1H), 1.47 (s, 2H), 1.25 (s, 18H), 1.01 – 0.84 (m, 9H); ^{13}C NNMR (125 MHz, $CDCl_3$) δ(ppm) 171.1, 156.5, 136.3, 128.5, 128.1, 127.9, 66.9, 60.6, 39.5, 31.9, 31.1, 29.6, 29.6, 29.5, 29.3, 29.3, 26.9, 22.7, 19.2, 18.0, 14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{42}N_2O_3^+$ $(M+H)^+$ (m/z): 419.3274; experimental $(M+H)^+$ (m/z): 419.3275; $[\alpha]_D$= -8.91 (25 °C, c= 0.0114 g/mL in $CHCl_3$).

Compound 2a. IR(ATR)(cm^{-1}) 3294, 2923, 2854, 1684, 1644, 1535, 1455, 1387, 1287, 1238, 1040; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.45 – 7.10 (m, 10H), 5.63 (s, 1H), 5.45 (m, 1H), 5.11 (d, *J* = 14.2 Hz, 2H), 4.34 (dd, *J* = 14.2, 7.6 Hz, 1H), 3.11 (m, 3H), 3.02(m, 1H), 1.36 (m, 2H), 0.79 (dd, *J* = 9.7, 5.2 Hz, 3H).; ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 170.9, 156.0, 136.7, 136.2, 129.3, 128.5, 128.1, 127.9, 126.9, 66.9, 56.5, 41.2, 39.0, 22.5, 11.3; HRMS (ESI-TOF)$^+$ calculated for $C_{20}H_{24}N_2O_3^+$ $(M+H)^+$ (m/z): 341.1865; experimental $(M+H)^+$ (m/z): 341.1864; $[\alpha]_D$= +3.60 (25 °C, c= 0.0111 g/mL in $CHCl_3$).

Compound 2b. IR(ATR)(cm^{-1}) 3295, 2921, 2853, 1687, 1647, 1532, 1456, 1384, 1289, 1233, 1038, ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm); 7.42 – 7.13 (m, 5H), 6.04 (s, 1H), 5.72 (d, *J* = 5.9 Hz, 1H), 5.06 (m, 2H), 4.40 (d, *J* = 7.0 Hz, 1H), 3.16 (dt, *J* = 20.2, 6.8 Hz, 1H), 3.06 (m, 3H), 1.48 – 1.05 (m, 12H), 0.90 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 170.6, 156.0, 141.1, 136.7, 136.2, 129.3, 128.5, 128.1, 127.9, 126.9, 66.9, 65.1, 56.5, 39.5, 39.0, 31.8, 29.2, 29.2, 26.8, 22.6, 14.1.; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{34}N_2O_3^+$ $(M+Na)^+$(m/z): 433.2467; experimental $(M+ Na)^+$(m/z): 433.2460; $[\alpha]_D$= +1.24 (25 °C, c= 0.0113 g/mL in $CHCl_3$).

Compound 2c. IR(ATR)(cm^{-1}) 3295, 2921, 2849, 1697, 1647, 1537, 1452, 1388, 1285, 1233, 1042; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.48 – 7.08 (m, 10H), 5.53 (s, 1H), 5.39 (s, 1H), 5.10 (s, 2H), 4.33 (d, *J* = 6.8 Hz, 1H), 3.12 (dd, *J* = 12.7, 6.4 Hz, 3H), 3.00 (dd, *J* = 13.6, 7.9 Hz, 1H), 1.43 – 1.03 (m, 18H), 0.89 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 170.4, 155.8 136.6, 136.2, 129.3, 128.7, 128.5, 128.2, 128.0, 127.0, 77.2, 77.0, 76.7, 67.0, 56.5, 39.5, 38.9, 31.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.3, 29.2, 26.7, 22.7, 14.1.; HRMS (ESI-TOF)$^+$ calculated for $C_{29}H_{42}N_2O_3^+$ $(M+H)^+$ (m/z): 467.3274; experimental $(M+H)^+$ (m/z): 467.3270; $[\alpha]_D$= -4.37 (25 °C, c= 0.0116 g/mL in $CHCl_3$).

General procedure for the synthesis of compounds 3a-c and 4a-c. In a 100 mL Erlenmeyer flask was added the corresponding amount of **1a-c** or **2a-c** over 20 mL of HBr/HAc under a N_2 atmosphere. The mixture was stirred until CO_2 formation ceased (1 hour). The content of the Erlenmeyer was poured into 100 mL of diethyl ether. The obtained solution was extracted with water (3x60 mL). The aqueous phase was washed with chloroform (3x20 mL). The aqueous phase was basified using NaOH till pH 12. The compound of interest was extracted with chloroform from this aqueous phase (3x80 mL). The organic phase was dried with $MgSO_4$, then filtered and the solvent was vacuum evaporated, the residue being dried under vacuum. A clear oil was obtained.

Compound 3a. IR(ATR)(cm^{-1}) 3295, 2965, 2921, 1647, 1539, 1452, 1380, 1345; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 3.22 (m, 3H), 2.27 (m, 1H), 1.51 (m, 2H), 1.26 (m, 2H), 1.97 (m, 3H), 0.91 (m, 3H), 0.79(m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 174.2, 60.2, 40.6, 30.8, 22.9, 19.7, 16.0, 11.4; HRMS (ESI-TOF)$^+$ calculated for $C_8H_{18}N_2O^+$ $(M+H)^+$ (m/z): 159.1497; experimental $(M+H)^+$ (m/z): 159.1494; $[\alpha]_D$= -51.21 (25 °C, c= 0.0119 g/mL in $CHCl_3$).

Compound 3b. IR(ATR)(cm^{-1}) 3299, 2961, 2921, 2853, 1647, 1536, 1464, 1372, 1345; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 3.24 (m, 3H), 2.29 (m, *J* = 6.9, *J* = 3.8 Hz, 1H), 1.46 (m, 2H), 1.39 – 1.17 (m, 12H), 0.96 (t, *J* = 7.0 Hz, 3H), 0.92 – 0.77 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 174.1, 60.2, 39.0, 31.8, 30.8, 29.7, 29.2, 29.2, 27.0, 22.6, 19.7, 16.0, 14.0; HRMS (ESI-TOF)$^+$ calculated for $C_{13}H_{28}N_2O^+$ $(M+H)^+$ (m/z): 229.2280; experimental $(M+H)^+$ (m/z): 229.2279; $[\alpha]_D$= -38.61 (25 °C, c= 0.0127 g/mL in $CHCl_3$).

Compound 3c. IR(ATR)(cm^{-1}) 3295, 2961, 2917, 2849, 1639, 1552, 1460, 1368; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 3.23 (m, 3H), 2.29 (m, *J* = 6.9, *J* = 3.8 Hz, 1H), 1.50 (m, *J* = 7.1 Hz, 2H), 1.44 – 1.18 (m, 18H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.93 – 0.71 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 174.1, 60.2, 39.0, 31.9, 30.8, 29.7, 29.6, 29.5, 29.3, 27.0, 22.6, 19.7, 16.0, 14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{17}H_{36}N_2O^+$ $(M+H)^+$ (m/z): 285.2906; experimental $(M+H)^+$ (m/z): 285.2908; $[\alpha]_D$= -23.17 (25 °C, c= 0.0111 g/mL in $CHCl_3$).

Compound 4a. IR(ATR)(cm^{-1}) 3291, 2965, 2929, 1647, 1524, 1456, 1380; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.31 – 7.16 (m, 5H), 3.55 (dd, *J* = 8.9, 4.1 Hz, 1H), 3.26 – 3.08 (m, 3H), 2.67 (dd, *J* = 13.7, 9.1 Hz, 1H), 1.70 (s, 2H), 1.46 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 174.2, 137.9, 129.3, 128.6, 126.7, 56.4, 41.1, 40.8, 22.7, 11.3; HRMS

(ESI-TOF)$^+$ calculated for $C_{12}H_{18}N_2O^+$ $(M+H)^+$ (m/z): 207.1497; experimental $(M+H)^+$ (m/z): 207.1499; $[\alpha]_D$= -15.28 (25 °C, c= 0.0127 g/mL in $CHCl_3$).

Compound 4b. IR(ATR)(cm^{-1}) 3295, 2957, 2921, 1647, 1524, 1456, 1380; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.41 – 7.12 (m, 5H), 3.59 (m, *J* = 10.4, *J* = 2.2 Hz, 1H), 3.32 – 3.16 (m, 3H), 2.70 (m, 1H), 1.46 (dd, *J* = 13.3, 6.7 Hz, 2H), 1.29 (s, 12H), 0.96 – 0.79 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 173.8, 137.9, 129.3, 128.7, 126.8, 56.4, 41.0, 39.1, 31.8, 29.5, 29.2, 29.2, 26.9, 22.6, 14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{17}H_{28}N_2O^+$ $(M+H)^+$(m/z): 277.2280; experimental $(M+H)^+$(m/z): 277.2274; $[\alpha]_D$= -26.88 (25 °C, c= 0.0127 g/mL in $CHCl_3$).

Compound 4c. IR(ATR)(cm^{-1}) 3287, 2953, 2917, 2853, 1631, 1552, 1520, 1468, 1364; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 7.35 – 7.21 (m, 5H), 3.60 (dd, *J* = 9.3, *J* = 4.1 Hz, 1H), 3.36 – 3.17 (m, 3H), 2.70 (dd, *J* = 13.7, *J* = 9.3 Hz, 1H), 1.48 (m, 2H), 1.43 – 1.10 (m, 18H), 0.89 (dd, *J* = 7.1, *J* = 6.1 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 174.0, 138.0, 129.3, 128.6, 126.7, 56.5, 41.1, 39.1, 31.9, 29.6, 29.6, 29.6, 29.5, 29.3, 29.28, 27.0, 22.7, 14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{21}H_{36}N_2O^+$ $(M+H)^+$ (m/z): 333.2906; experimental $(M+H)^+$ (m/z): 333.2905; $[\alpha]_D$= -49.57 (25 °C, c= 0.0127 g/mL in $CHCl_3$).

General procedure for the synthesis of compounds 5a-c and 6a-c. 4-carboxybenzaldehyde was dissolved in 50 mL of dichloromethane into a two-necked round bottom flask. The round bottom flask was connected to a reflux condenser and the setup was placed in an ice bath under a N_2 atmosphere. 2.5 equivalents of $SOCl_2$ were added dropwise and the resulting mixture stirred for 10 minutes. Then, 1 equivalent of the compound **3a-c** or **4a-c** dissolved in dichloromethane was added. After adding the amine, the reaction mixture was heated at 65 °C for 2 hours and the stirring was continued at room temperature overnight. After this time, the reaction mixture was neutralized with $NaHCO_3$. The product was extracted with dichloromethane (3x80 mL). The organic phases were collected and dried with

$MgSO_4$. The solvent was vacuum distilled and the product was purified by flash chromatography using a gradient concentration of hexane-ethyl acetate.

Compound 5a. IR(ATR)(cm^{-1}) 3293, 2962, 1702, 1623, 1530; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.02 (s, 1H), 7.94 (dd, *J* = 8.3, *J* = 2.7 Hz, 2H), 7.85 (dd, *J* = 7.9, *J* = 5.9 Hz, 2H), 7.78 (d, *J* = 6.7 Hz, 1H), 7.21 (d, *J* = 4.3 Hz, 1H), 4.59 (t, *J* = 8.5 Hz, 1H), 3.29 (m, *J* = 10.1, *J* = 6.7, *J* = 3.5 Hz, 1H), 3.10 (m, *J* = 7.1, *J* = 1.7 Hz, 1H), 2.24 (m, *J* = 7.0 Hz, 1H), 1.50 (m, 2H), 1.04 (m, 6H), 0.88 (m, *J* = 3.7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.5, 171.5, 166.5, 139.2, 138.2, 129.6, 128.0, 59.7, 41.3, 31.2, 22.6, 19.3, 18.9, 11.4; HRMS (ESI-TOF)$^+$ calculado para $C_{16}H_{22}N_2O_3^+$ $(M+H)^+$ (m/z): 291.1709; experimental $(M+H)^+$ (m/z): 291.1704; $[\alpha]_D$= -0.94 (25 ˚C, c= 0.0053 g/mL in $CHCl_3$).

Compound 5b. IR(ATR)(cm^{-1}) 3289, 2962, 1695, 1623, 1533; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.01 (s, 1H), 7.95 – 7.79 (m, 4H), 7.03 (d, *J* = 8.5 Hz, 1H), 6.14 (t, *J* = 5.5 Hz, 1H), 4.38 (dd, *J* = 8.6, *J* = 7.3 Hz, 1H), 3.27 (m, 7 *J* =.2 Hz, 1H), 3.13 (m, *J* = 5.6 Hz, 1H), 2.14(m, 1H), 1.44 (m, 2H), 1.18 (m, 10H), 0.97 (dd, *J* = 6.8, *J* = 0.9 Hz, 6H), 0.80 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.4, 171.2, 166.4, 139.3, 138.3, 129.6, 127.9, 59.6, 39.6, 31.7, 31.4, 29.4, 29.2, 29.1, 26.9, 22.6, 19.3, 18.8, 14.0; HRMS (ESI-TOF)$^+$ calculated for $C_{21}H_{32}N_2O_3^+$ $(M+H)^+$ (m/z): 361.2491; experimental $(M+H)^+$ (m/z): 361.2490; $[\alpha]_D$= + 5.07 (25 °C, c= 0.0043 g/mL in $CHCl_3$).

Compound 5c. IR(ATR)(cm^{-1}) 3285, 2920, 1699, 1627, 1533; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.03 (m, 1H), 7.92 (t, *J* = 10.3 Hz, 2H), 7.86 (m, 2H), 7.52 (d, *J* = 8.7 Hz, 1H), 7.01 (t, *J* = Hz, 1H), 4.57 (t, *J* = 8.3 Hz, 1H), 3.31 (m, 1H), 3.12 (m, *J* = 6.4 Hz, 1H), 2.22 (m, 1H), 1.46 (m, 2H), 1.25 (m, 18H), 1.01 (m, 6H), 0.86 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.3, 171.1, 166.4, 139.3, 138.3, 129.6, 127.9, 59.5, 39.6, 31.9, 31.4, 29.6, 29.6, 29.5,

29.5, 29.4, 29.3, 29.2, 26.9, 22.6, 19.3, 18.8, 14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{40}N_2O_3^+$ $(M+H)^+$ (m/z): 417.3117; experimental $(M+H)^+$ (m/z): 417.3117; $[\alpha]_D$= +7.37 (25 °C, c= 0.009 g/mL in $CHCl_3$).

Compound 6a. IR(ATR)(cm^{-1}) 3314, 2962, 1695, 1631, 1526; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.09 (m, 1H), 7.92 (q, *J* = 8.2 Hz, 4H), 7.29 (m, 6H), 5.69 (s, 1H), 4.81 (m, 1H), 3.29 (dd, *J* = 13.5, *J* = 5.8 Hz, 1H), 3.18 (m, *J* = 6.6 Hz, 1H), 3.09 (m, 2H), 1.37 (m, 2H), 0.81 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.4, 191.4, 170.7, 166.0, 138.9, 138.3, 136.6, 129.7, 129.3, 128.7, 127.8, 127.1, 55.5, 41.3, 38.9, 22.5, 11.2; HRMS (ESI-TOF)$^+$ calculated for $C_{20}H_{22}N_2O_3^+$ $(M+H)^+$ (m/z): 339.1709; experimental $(M+H)^+$ (m/z): 339.1706; $[\alpha]_D$= +7.52 (25 °C, c= 0.0085 g/mL in $CHCl_3$).

Compound 6b. IR(ATR)(cm^{-1}) 3301, 2926, 1695, 1634, 1535; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.07 (d, *J* = 3.7 Hz, 1H), 8.93 (m, 4H), 7.27 (m, 6H), 5.85 (t, *J* = 5.5 Hz, 1H), 5.83 (m, 1H), 3.28 (dd, *J* = 13.5, *J* = 5.9 Hz, 1H), 3.20 (m, 1H), 3.11 (m, 2H), 1.39 – 1.10 (m, 12H), 0.89 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.4, 170.4, 165.9, 138.9, 138.3, 136.6, 129.7, 129.3, 128.7, 127.8, 127.1, 55.5, 39.7, 39.0, 31.7, 29.2, 29.2, 29.1, 26.8, 22.6, 14.0; HRMS (ESI-TOF)$^+$ calculated for $C_{25}H_{32}N_2O_3^+$ $(M+H)^+$ (m/z): 409.2491; experimental $(M+H)^+$ (m/z): 409.2488; $[\alpha]_D$= -3.46 (25 °C, c= 0.0107 g/mL in $CHCl_3$).

Compound 6c. IR(ATR)(cm^{-1}) 3300, 2923, 1695, 1631, 1533; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 10.06 (d, *J* = 0.8 Hz, 1H), 8.90 (m, 4H), 7.28 (m, 6H), 5.92 (t, *J* = 5.6 Hz, 1H), 4.84 (dd, *J* = 14.1, *J* = 8.0 Hz, 1H), 3.36 – 2.98 (m, 4H), 1.54 – 0.95 (m, 20H), 0.88 (dt, *J* = 7.0, *J* = 3.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm) 191.4, 170.4, 165.9, 138.9, 138.4, 136.7, 129.7, 129.3, 128.7, 127.8, 127.1, 55.5, 39.7, 39.0, 31.9, 29.6, 29.6, 29.5, 29.3, 29.2, 29.2, 26.8, 22.7,

14.1; HRMS (ESI-TOF)$^+$ calculated for $C_{29}H_{40}N_2O_3^+$ $(M+H)^+$ (m/z): 465.3117; experimental $(M+H)^+$ (m/z): 465.3113; $[\alpha]_D$= -0.94 (25 °C, c= 0.0054 g/mL in $CHCl_3$).

General procedure for the synthesis of compound 7. 4-Methoxylacetophenone was added to 1 equivalent of acetic anhydride. Then 2 equivalents of BF_3•OEt_2 were added. The solution got dark and then the system was refluxed for 75 minutes. After this time, the reaction mixture was cooled to room temperature and poured into excess ether (200 mL). The obtained red precipitate was filtered, washed with ether and dried under vacuum.

Compound 7. (1.5 g, 32%) IR (ATR)(cm^{-1}): 3086, 2943, 2849, 1640, 1593, 1498; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.85 (s, 1H), 8.45 (m, 4H), 8.38 (s, 1H), 7.28 (m, 4H), 3.95 (d, *J* = 7.1 Hz, 6H), 2.89 (s, 3H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 174.7, 170.5, 166.0, 165.2, 163.1, 132.8, 131.3, 124.5, 121.7, 116.1, 115.8, 115.6, 111.9, 56.6, 56.5, 56.5, 21.3; HRMS(ESI-TOF)$^+$ calculated for $C_{20}H_{19}O_3^+$ (M^+)(m/z): 307.1334; experimental (M^+)(m/z): 307.1341.

General procedure for the synthesis of compounds 8a-c and 9a-c. The corresponding 2, 4-diaryl-6-pyrylium salt and 1.1 equivalents of the selected compound **5a-c** or **6a-c** were mixed in a round bottom flask in the presence of 10 mL of acetic acid. The reaction mixture was refluxed overnight. Then, the obtained solution was cooled to room temperature and poured into excess ether (150 mL). The observed precipitate was filtered, washed with ether and dried under vacuum.

Compound 8a. IR(ATR)(cm^{-1}) 2959, 2848, 1631, 1583, 1488, 1249, 1173; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.81 (d, *J* = 19.9 Hz, 1H), 8.58 (m, 3H), 8.48 (d, *J* = 8.3 Hz, 2H), 8.37 (d, *J* = 8.7 Hz, 1H), 8.31 (d, *J* = 16.3 Hz, 1H), 8.06 (d, *J* = 8.2 Hz, 3H), 7.97 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 16.4 Hz, 1H), 7.30(m, 4H), 4.29 (t, *J* = 8.4 Hz, 1H), 3.96 (d, *J* = 9.3 Hz, 6H), 3.11 (dd, *J*

= 13.0, *J* = 6.2 Hz, 1H), 3.00 (dd, *J* = 12.8, *J* = 6.0 Hz, 1H), 2.12(m, 1H), 1.44 (dd, *J* = 13.1, *J* = 7.2 Hz, 2H), 0.97(m, 6H), 0.86 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 171.2, 169.1, 167.5, 166.0, 165.9, 165.3, 162.2, 142.5, 137.4, 136.8, 132.6, 131.7, 129.0, 128.9, 124.9, 121.9, 120.8, 116.1, 115.8, 115.1, 59.7, 56.7, 56.6, 30.6, 22.7, 21.5, 19.8, 19.4, 11.9; HRMS (ESI-TOF)$^+$ calculated for $C_{36}H_{39}N_2O_5^+$ (M^+)(m/z): 579.2859; experimental (M^+)(m/z): 579.2860; $[\alpha]_D$= -229.10 (25 °C, c= 0.0067 g/mL in CH_3CN).

Compound 8b. IR(ATR)(cm^{-1}) 2935, 2854, 1635, 1587, 1484, 1249, 1173; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.82 (s, 1H), 8.58 (m, 3H), 8.48 (t, *J* = 7.9 Hz, 2H), 8.37 (d, *J* = 8.6 Hz, 1H), 8.31 (d, *J* = 16.3 Hz, 1H), 8.04 (dd, *J* = 11.6, *J* = 6.8 Hz, 3H), 7.97 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 16.3 Hz, 1H), 7.29 (m, 4H), 4.28 (t, *J* = 8.5 Hz, 1H), 3.95 (m, 6H), 3.13 (m, 6.5 Hz, 1H), 3.02 (m, *J* = 5.8 Hz, 1H), 2.11(m, 1H), 1.40 (d, *J* = 6.8 Hz, 2H), 1.23 (d, *J* = 7.4 Hz, 10H), 0.97 (m, 6H), 0.84 (dd, *J* = 6.8, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 172.4, 171.1, 169.1, 167.5, 166.0, 165.9, 165.3, 162.2, 142.4, 137.4, 136.8, 132.6, 131.7, 129.0, 128.9, 124.9, 121.9, 120.7, 116.1, 115.8, 115.1, 59.7, 56.7, 56.5, 31.6, 30.6, 29.4, 29.1, 26.8, 22.5, 21.5, 19.8, 19.4, 14.4.; HRMS (ESI-TOF)$^+$ calculated for $C_{41}H_{49}N_2O_5^+$ (M^+)(m/z): 649.3641; experimental (M^+)(m/z): 649.3635; $[\alpha]_D$= -311.89 (25 ˚C, c= 0.0067 g/mL in CH_3CN).

Compound 8c. IR(ATR)(cm^{-1}) 2925, 2854, 1635, 1583, 1492, 1253, 1173; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.84 (s, 1H), 8.60 (m, 3H), 8.48 (d, *J* = 8.8 Hz, 2H), 8.37 (d, *J* = 8.6 Hz, 1H), 8.32 (d, *J* = 16.3 Hz, 1H), 8.04 (dd, *J* = 16.5, *J* = 6.9 Hz, 3H), 7.97 (d, *J* = 8.2 Hz, 2H), 7.74 (d, *J* = 16.3 Hz, 1H), 7.30 (dd, *J* = 12.2, *J* = 9.0 Hz, 4H), 4.28 (t, *J* = 8.4 Hz, 1H), 3.98 (m, 6H), 3.13 (dd, *J* = 13.1, 6.5 Hz, 1H), 3.02 (dd, *J* = 12.3, *J* = 5.6 Hz, 1H), 2.12 (dd, *J* = 14.0, *J* = 6.8 Hz, 1H), 1.40 (d, *J* = 6.8 Hz, 2H), 1.23 (d, *J* = 9.6 Hz, 18H), 0.93 (m, 6H), 0.83 (dd, *J* = 7.2, *J* = 6.4 Hz, 3H).; ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 172.4, 171.2, 169.1, 167.5, 166.0, 165.9, 165.3, 162.2, 142.5, 137.4, 136.8, 132.6, 131.6, 129.0, 128.9, 124.9, 121.9, 120.8, 116.1, 115.8,

115.1, 59.7, 56.7, 56.6, 31.7, 30.6, 29.5, 29.5, 29.4, 29.4, 29.2, 29.1, 26.8, 22.5, 21.5, 19.8, 19.4, 14.4; HRMS (ESI-TOF)$^+$ calculated for $C_{45}H_{57}N_2O_5^+$ (M^+)(m/z): 705.4267; experimental (M^+)(m/z): 705.4264; $[\alpha]_D$= -76.93 (25 °C, c= 0.0088 g/mL in CH_3CN).

Compound 9a. IR(ATR)(cm^{-1}) 2930, 2853, 1634, 1588, 1485, 1253, 1181; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.83(m, 1H), 8.69 (d, *J* = 8.5 Hz, 1H), 8.60 (t, *J* = 8.3 Hz, 2H), 8.56 (s, 1H), 8.48 (t, *J* = 9.3 Hz, 2H), 8.29 (d, *J* = 16.3 Hz, 1H), 8.07 (dd, *J* = 15.3, *J* = 9.6 Hz, 1H), 8.96 (m, 4H), 7.72 (d, *J* = 16.3 Hz, 1H), 7.30 (m, *J* = 15.5, *J* = 7.5 Hz, 8H), 7.17 (m, 1H), 4.72(m, 1H), 3.95 (m, 6H), 3.05 (m, 4H), 1.41 (dq, *J* = 14.2, *J* = 7.2 Hz, 2H), 0.83 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 172.4, 171.4, 169.1, 167.5, 165.9, 165.8, 165.3, 162.2, 142.4, 138.8, 137.6, 136.6, 132.6, 131.6, 129.6, 129.0, 128.8, 128.5, 126.7, 124.9, 121.9, 120.8, 116.1, 115.8, 115.1, 56.6, 56.6, 55.5, 38.0, 22.7, 21.5, 11.8; HRMS (ESI-TOF)$^+$ calculated for $C_{40}H_{39}N_2O_5^+$ (M^+)(m/z): 627.2859; experimental (M^+)(m/z): 627.2852; $[\alpha]_D$= -257.14 (25 °C, c= 0.007 g/mL in CH_3CN).

Compound 9b. IR(ATR)(cm^{-1}) 2933, 2849, 1635, 1587, 1488, 1253, 1173; ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ(ppm) 8.83(m, 1H), 8.68 (d, *J* = 8.4 Hz, 1H), 8.59 (m, 2H), 8.56 (d, *J* = 1.3 Hz, 1H), 8.49 (t, *J* = 9.7 Hz, 2H), 8.30 (d, *J* = 16.3 Hz, 1H), 8.06 (t, *J* = 5.6 Hz, 1H), 7.95 (m, 4H), 7.72 (d, *J* = 16.3 Hz, 1H), 7.29 (m, 8H), 7.17 (m, 1H), 4.71(m, 1H), 4.95 (m, 6H), 3.11 – 2.98 (m, 4H), 1.37 (dd, *J* = 13.8, *J* = 7.8 Hz, 2H), 1.29 – 1.16 (m, 10H), 0.85 (m, 3H); ^{13}C NMR (125 MHz, DMSO-*d*$_6$) δ(ppm) 172.4, 171.3, 169.1, 167.5, 165.9, 165.8, 165.3, 162.2, 142.4, 138.8, 137.5, 136.6, 132.6, 131.7, 129.6, 129.0, 128.8, 128.6, 128.5, 126.7, 124.9, 121.9, 120.8, 116.1, 115.8, 115.1, 56.7, 56.6, 55.5, 31.7, 29.5, 29.2, 29.1, 26.8, 22.5, 21.5, 14.4; HRMS (ESI-TOF)$^+$ calculated for $C_{45}H_{49}N_2O_5^+$ (M^+)(m/z): 697.3641; experimental (M^+)(m/z): 697.3639; $[\alpha]_D$= -326.47 (25 °C, c= 0.0077 g/mL in CH_3CN).

Compound 9c. IR(ATR)(cm^{-1}) 2929, 2849, 1631, 1587, 1483, 1253, 1177; ^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm) 8.83 (t, *J* = 13.3 Hz, 1H), 8.68 (d, *J* = 8.4 Hz, 1H), 8.59 (m, 2H), 8.57 (s, 1H), 8.48 (d, *J* = 8.9 Hz, 2H), 8.30 (d, *J* = 16.3 Hz, 1H), 8.05 (m, 1H), 7.96 (q, *J* = 8.7 Hz, 4H), 7.73 (d, *J* = 16.3 Hz, 1H), 7.37 – 7.21 (m, 8H), 7.16 (m, 1H), 4.70 (m, 1H), 3.97 (m, 6H), 3.04 (m, 4H), 1.36 (d, *J* = 12.7 Hz, 2H), 1.24 (m, 18H), 0.82 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ(ppm) 172.4, 171.3, 169.1, 167.5, 165.9, 165.8, 165.3, 162.2, 142.4, 138.8, 137.4, 136.5, 132.6, 131.6, 129.6, 128.9, 128.8, 128.7, 128.5, 126.8, 124.9, 121.9, 120.8, 116.1, 115.8, 56.7, 56.6, 55.4, 31.7, 29.5, 29.5, 29.4, 29.2, 26.7, 22.5, 21.5, 14.4; HRMS (ESI-TOF)$^{+}$ calculated for $C_{49}H_{57}N_2O_5^{+}$ (M^{+}) (m/z): 753.4267; experimental (M^{+})(m/z): 753.4262; [α]D= -360 (25 °C, c= 0.0067 g/mL in CH_3CN).

Fluorimetric and absorption studies.

Photophysical characterization. Emission and absorption spectra of all compounds (10 μM) were recorded on solvents of different polarity (dichloromethane, acetonitrile and phosphate buffered saline solution (PBS 20 mM). The steady-state spectra and emission decays of the corresponding solutions were recorded using 1 x 1 cm quartz cells.

Fluorescence titrations with N-protected amino acids. 1 mM stock solution in acetonitrile was prepared for each of the compounds **8a-c** and **9a-c**. From this solution, a diluted one (10μM) was prepared in the fluorescence cuvette. To this solution, aliquots of a 0.5 M stock solution of the corresponding N-protected amino acid in acetonitrile were added. Emission spectra were recorded exciting at the absorption maximum wavelength of the dye.

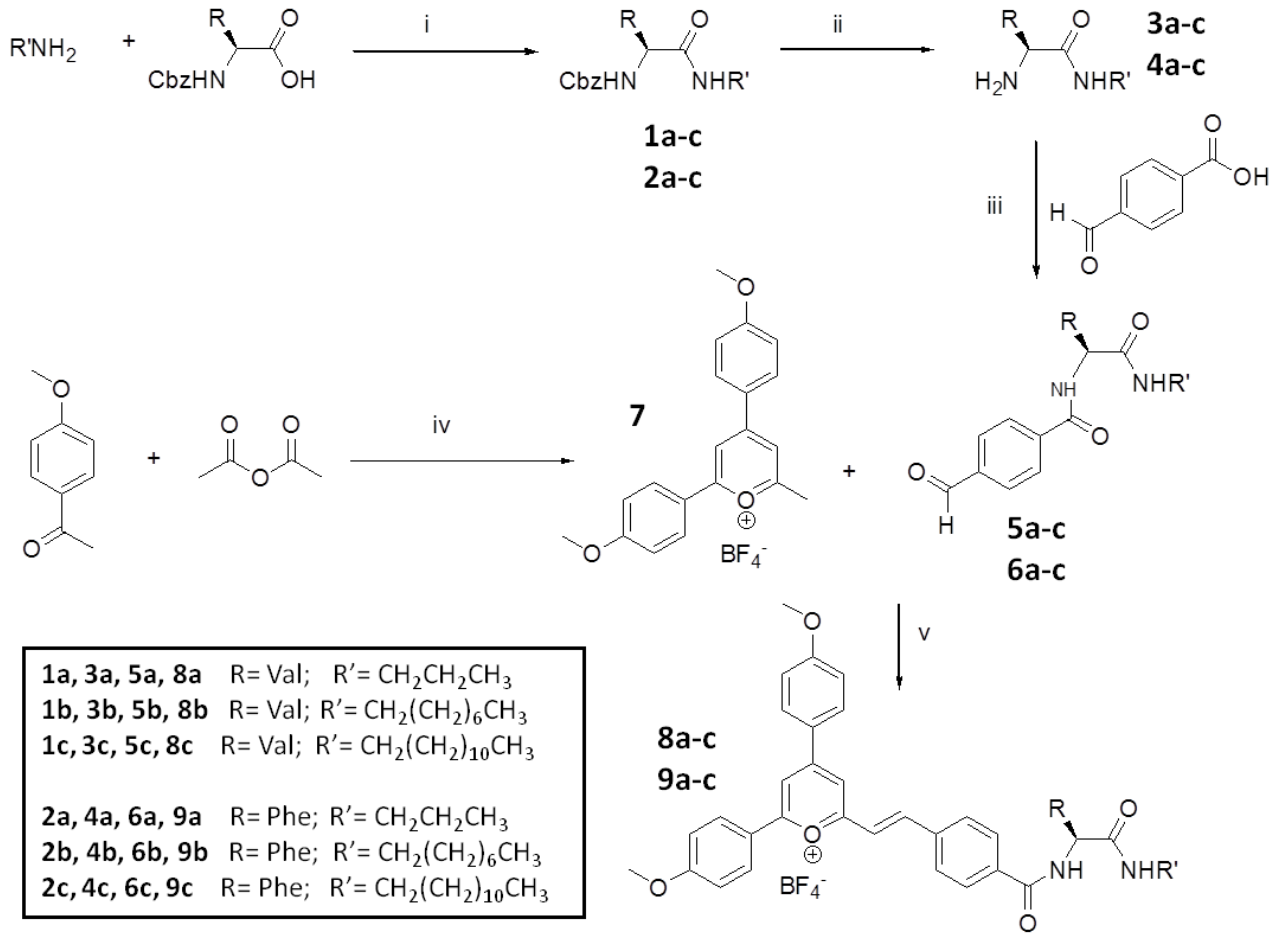
^{1}H NMR titrations.

2 mL of a 5 mM solution of compound **8a** was prepared in deuterated acetonitrile (solution a). 1 mL of this solution was used to prepare a 0.5 M solution of the corresponding N-protected amino acid in deuterated acetonitrile (solution b). 0.7 mL of solution a were placed in an NMR tube and increasing aliquots of solution b were added until a final concentration of 190 mM of amino acid. ^{1}H NMR spectra were recorded after each addition of substrate.

Results and discussion

Six new pyrylium salts (**8a-c** and **9a-c**) were prepared according to Scheme 1. They contain a styrylpyrylium core fluorophore and a pending arm displaying an amino acid (valine or phenylalanine) fragment and an alkyl chain of variable length (3, 8 or 12 carbon atoms). While the presence of the amino acid moiety provides a number of recognition sites, and the potential for enantioselective recognition, like in previously reported receptors,[15–17] the long alkyl chain can be expected to contribute to the generation of hydrophobic interactions, since these could play a key role in the complexation process and is currently a topic of intense research.[42–44]

The synthesis of the intermediate **7** was carried out according to literature procedures.[45] For the preparation of intermediates **5a-c** and **6a-c**, the first step was the coupling between the N-protected amino acid (Z-Val-OH or Z-Phe-OH) and the corresponding alkylamine to give **1a-c** and **2a-c**.[46] Cbz deprotection was achieved in acidic medium to yield **3a-c** and **4a-c**.[47] The resulting amido amines were reacted with 4-formylbenzoic acid in the presence of $SOCl_2$ to give the diamides **5a-c** and **6a-c**.[48] Finally, the obtained aldehyde was condensed with the 2,4-diaryl-6-methylpyrylium salt obtained previously (**7**) by refluxing the mixture in the presence of acetic acid and giving rise to the desired pseudopeptidic styryl pyryliums **8a-c** and **9a-c**.[49] The identity of compounds **8a-c** and **9a-c** was confirmed by ^{1}H / ^{13}C NMR spectroscopy and HRMS spectrometry data.



Scheme 1. Synthetic route to obtain pseudopeptidic styryl pyrylium salts **8a-c** and **9a-c**. Reagents and conditions: i) Et_3N, ClCOOEt in THF (24 h, rt); ii) HBr/CH_3COOH (1 h, rt); iii) $SOCl_2$ in CH_2Cl_2 (2 h at 65 ºC, then 24 h at rt); iv) $BF_3{\cdot}OEt_2$ (75 min, 50-120 ºC); v) CH_3COOH (24 h, reflux).

Compounds **8a-c** and **9a-c** were fully characterized in acetonitrile (ACN), dichloromethane (DCM) and PBS (20mM) using UV-Vis and fluorescence spectroscopies. Figures 1 and 2 present the absorption and emission spectra of **8a-c** and **9a-c** in several solvents, while Table 1 gathers the data collected for all compounds, including absorption (λ_{abs}) and emission maxima (λ_f), extinction coefficients (ε), emission quantum yields (ϕ_f) and fluorescence lifetimes (τ_f). The solvatochromic effect is very small for all the compounds irrespective of the amino acid or the length of the alkyl chain, since the absorption and emission features for all the compounds are very similar, thus indicating a small charge-transfer character of the excited state. The effect of the solvent is more pronounced in the emission quantum yield, since in DCM all compounds are strongly fluorescent, with ϕ_f =0.45-0.51, but in polar solvents this value drops with ϕ_f=0.14-0.19 in ACN and ϕ_f =0.01-0.03 in PBS. The radiative deactivation constants

calculated with the emission quantum yields and fluorescence lifetimes ($k_r = \phi_f / \tau_f$) resulted higher in apolar DCM (ca. $1.0 \cdot 10^8$ s^{-1}- $1.2 \cdot 10^8$ s^{-1}) that in polar ACN (ca. $6 \cdot 10^7$ s^{-1}- $1.0 \cdot 10^8$ s^{-1}) or PBS (ca. $6 \cdot 10^6$ s^{-1}- $1.3 \cdot 10^7$ s^{-1}). This higher k_r in apolar solvents is in agreement with the data reported by Haucke et al. for other pyrylium compounds.[50] In some instances, the introduction of an extended conjugation in pyrylium compounds can lead to a dual emission, as it has been studied previously.[33,51,52] However, this is not the case for compounds **8a-c** and **9a-c**, which display a single emission band. Structurally related pyrylium dyes with *p*-methoxystyryl units display a dual emission with a localized emission at *ca*. 540 nm and a delocalized fluorescence at *ca*.620 nm. The fluorescence observed for **8a-c** and **9a-c** arises from a localized state centered at the pyrylium core since the emission maxima is at 537-560 nm in all the cases, with no contribution from any other band above 600 nm. The reason for this difference can be accounted for taking into account the electron-withdrawing nature of the carboxamide group linking the pyrylium fluorophore to the amino acid part of the molecule. In the case of the above mentioned pyrylium salts with dual emissive behaviour, the group present at this position is an electron-donating alkoxy one, which enhances the charge-transfer nature of the excited state, and hence favouring the occurrence of fluorescence from a delocalized state.

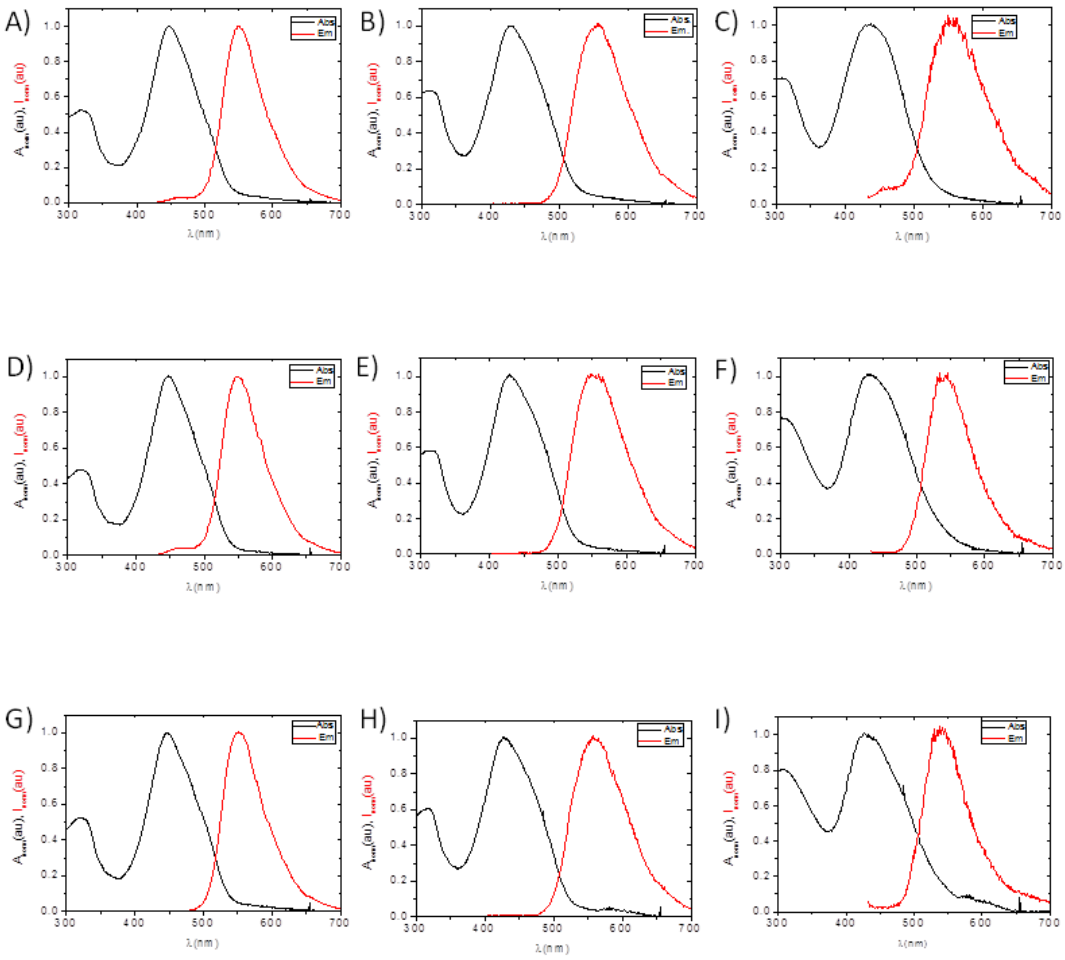


Figure 1 Normalized absorption (black) and emission (red) spectra for **8a**-**8c** in dichlorometane (left column), acetonitrile (central column) and PBS 20 mM (right column). A, B, C: **8a**; D, E, F: **8b**; G, H, I: **8c**. λ_{exc} = 400 nm.

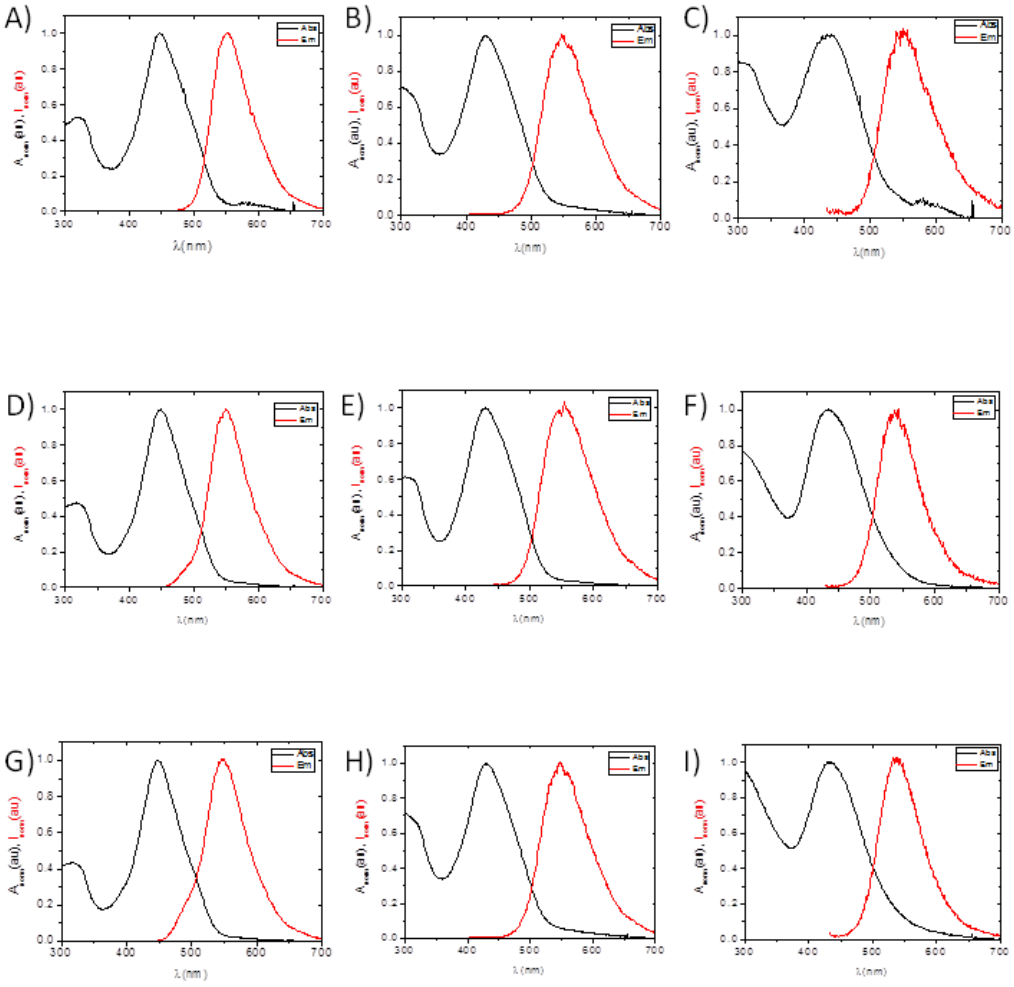


Figure 2. Normalized absorption (black) and emission (red) spectra for **9a-9c** in dichlorometane (left column), acetonitrile (central column) and PBS 20 mM (right column). A, B, C: **9a;** D, E, F: **9b**; G, H, I: **9c**. λ_{exc} = 400 nm.

A series of fluorescence titrations were carried out to study the interaction of these pseudopeptidic pyrylium with N-protected amino acids. Ala, Val, Phe, Met, Trp and Tyr, in their N-protected forms and L-configuration were initially selected. For this purpose, the absorption and emission spectra of **8a-c** and **9a-c** were recorded in the presence of increasing amounts of the corresponding substrate (see Figure 3 for the case of **8a**). For those amino acid derivatives presenting a measurable interaction, titrations were also carried out with the appropriate D-enantiomer. Amino acids with alkyl side chains (Z-Ala-OH and Z-Val-OH) or a benzene ring (Z-Phe-OH) did not induce changes in the absorption or emission spectra. By contrast, in the experiments with amino acids displaying electron rich side chains (Z-Met-OH, Z-Tyr-OH and Z-Trp-OH) a decrease in the intensity of the emission was observed.

Table 1 Photophysical properties of compounds **8a-c** and **9a-c** in acetonitrile (A), dichloromethane (D) and PBS 20mM (W).

Comp.	Solvent	λ_{abs} (nm)	log ε	λ_f(nm)	Φ_f	τ_f (ns)	k_r (s^{-1}) / 10^7
8a	D	448	4.76	550	0.48	4.4	10.9
	A	430	4.74	556	0.17	2.4	7.1
	W	437	4.55	554	0.03	2.3	1.3
8b	D	448	4.77	550	0.51	4.3	11.9
	A	428	4.71	553	0.19	1.9	10
	W	438	4.55	541	0.03	3.1	1.0
8c	D	448	4.77	552	0.50	4.4	11.4
	A	428	4.75	560	0.16	1.7	9.4
	W	433	4.58	540	0.02	3.8	0.6
9a	D	448	4.79	551	0.45	4.4	10.2
	A	428	4.71	557	0.14	2.2	6.4
	W	433	4.56	549	0.03	2.4	1.2
9b	D	449	4.80	550	0.46	4.4	10.4
	A	430	4.76	553	0.17	2.4	7.1
	W	433	4.59	538	0.03	3.3	0.9
9c	D	448	4.63	547	0.49	4.2	11.7
	A	430	4.71	548	0.15	2.6	5.8
	W	433	4.52	537	0.03	3.3	0.9

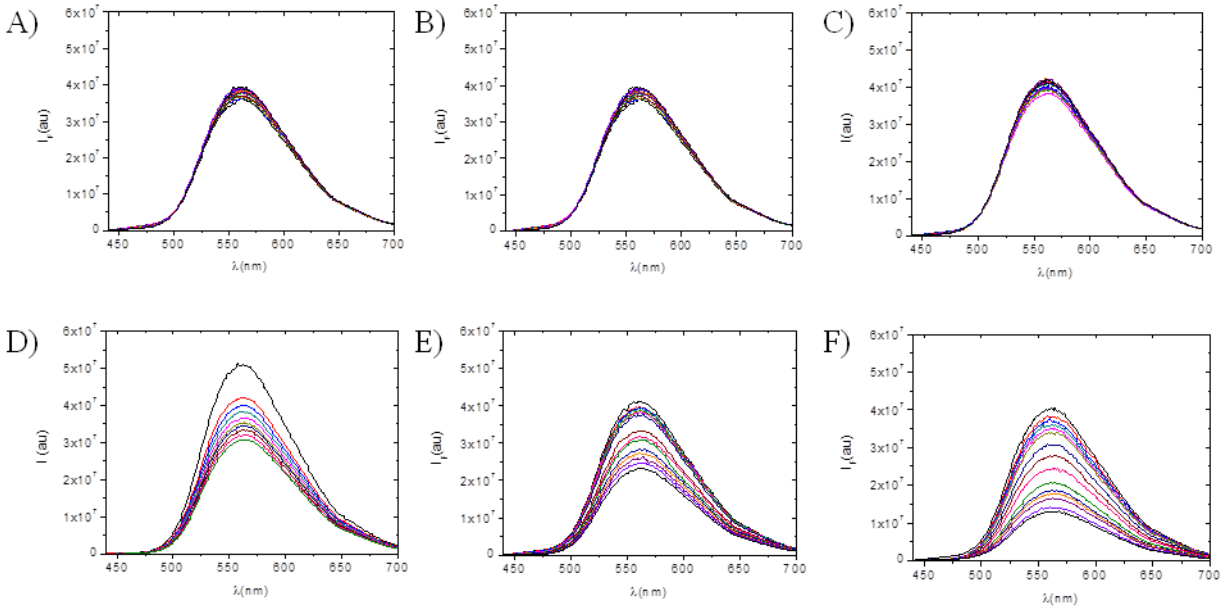


Figure 3. Fluorescence spectra of **8a** in the presence of increasing amounts of N-protected amino acids (from 0 to 45 mM): A) Z-L-Ala-OH, B) Z-L-Val-OH, C) Z-L-Phe-OH, D) Z-L-Met-OH, E) Z-L-Tyr-OH and F) Z-L-Trp-OH.

The quenching of the fluorescence can occur via dynamic or static mechanisms.[19] The static mechanism involves a supramolecular association between the receptor and the substrate, whereas the dynamic mechanism implies only collisional interactions with the excited state. Several models have been suggested to describe both types of quenching. The simplified expression shown in eqn (1) has been habitually used to describe the fluorescence quenching when both mechanisms are operating.[53]

$$I_0/I = (1 + K_d \cdot[Q])(1 + K_a \cdot[Q]) \quad (1)$$

In eqn (1) the ratio of emission intensities in the absence (I_0) and in the presence (I) of a certain quencher Q depends on its concentration ([Q]), and K_d and K_a are the constants describing the dynamic and static processes, respectively. When only collisional quenching takes place then the expression can be written as eqn (2), where k_q is the bimolecular quenching constant.

$$I_0/I = \tau_0/\tau = 1 + K_d \cdot[Q] = 1 + k_q \cdot \tau_f \cdot[Q] \quad (2)$$

In our case tryptophan is the substrate causing the greatest decrease in fluorescence when interacting with all the studied pyrylium salts (Figures 2 and 3). Fluorescence quenching in the case of Z-Met-OH and Z-Tyr-OH is notably lower than in the case of Trp. In most cases, quenching fits well (Figure 4) to a linear Stern-Volmer model, indicating that dynamic quenching is the only contribution. An upward deviation from linearity is observed, however, for the fluorescence quenching of **8a** by Z-Trp-OH, which is indicative of the presence of static quenching processes due to the formation of ground state complexes.

Table 2 summarizes the calculated K_d values for the quenching of all pseudopeptidic pyrylium salts by different N-protected amino acids. As it can be seen, the most effective quencher is tryptophan, with constants between 29.6 and 40.5 M^{-1}. Z-Tyr-OH and Z-Met-OH induce quenching with constants between 5.9 and 19.9 M^{-1}. The observed quenching can be rationalized through a photoinduced electron transfer (PeT) process taking place from the electron-rich side chain to the excited state (S_1) of the pyrylium salt. This process is favoured in the case of Trp having a lower oxidation potential than the other amino acids. The quenching

effect of several amino acids (or related compounds) has been studied for other systems[12,18,19,54,55] and in all the cases PeT has been found the key to explain the pronounced tendency of Trp to cause the highest quenching effect. Other substances with electron-donating properties have been also reported to cause quenching of the fluorescence of pyrylium dyes.[56,57]

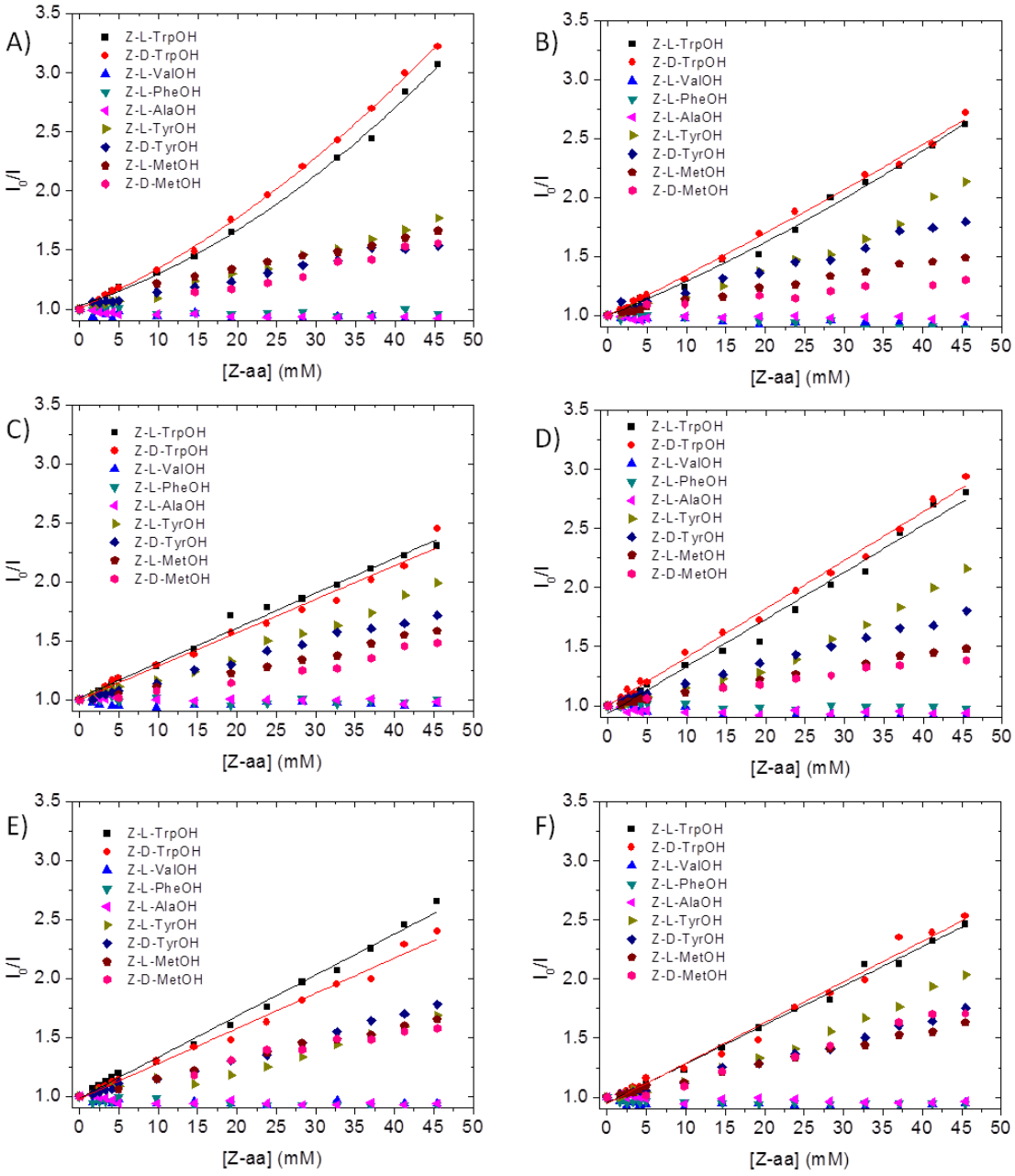


Figure 4. Stern-Volmer plots for the studied interactions between compounds A) **8a**, B) **8b**, C) **8c**, D) **9a**, E) **9b** and F) **9c** and the different N-protected amino acids.

Table 2. K_d values (M^{-1}) for the quenching of the fluorescence of compounds **8a-c** and **9a-c** in the presence of different N-protected amino acids in acetonitrile.

Aminoacid	8a	8b	8c	9a	9b	9c
Z-L-Trp-OH	30.6 ±1.3	29.8 ± 0.3	34.8 ± 0.8	35.4 ± 0.9	40.5 ± 1.0	33.0 ± 0.6
Z-D-Trp-OH	33.5 ±0.7	26.6 ± 0.5	30.4 ± 0.7	37.0 ± 0.9	41.0 ± 0.8	33.6 ± 0.7
Z-L-Tyr-OH	15.8 ± 0.5	19.4 ± 0.7	13.1 ± 0.6	19.9 ± 0.7	16.5 ± 1.0	17.7± 0.1
Z-D-Tyr-OH	11.7 ± 0.2	16.3 ± 0.3	16.9 ± 0.3	17.5 ±0.5	16.9 ± 0.3	16.2 ± 0.4
Z-L-Met-OH	13.5 ± 0.6	13.1 ± 0.4	14.5 ± 0.4	11.0 ± 0.2	10.9 ± 0.1	13.8 ± 0.2
Z-D-Met-OH	12.9 ± 0.9	10.8 ± 0.7	12.8 ± 0.9	5.9 ± 0.5	8.6 ± 0.4	17.4 ± 0.7

Notably, **8a** is the only receptor showing this moderate association since **8b**, **8c** and **9a-c** presented linear fits. The lack of curvature of the corresponding Stern-Volmer plots is indicative of a pure collisional mechanism for the quenching in these cases. The association constants (K_a) in the ground state could be roughly estimated from the quenching data for the interaction of **8a** with the L ($5.3 \pm 0.4\ M^{-1}$) and D ($5.5 \pm 0.3\ M^{-1}$) enantiomers of Z-Trp-OH applying eqn (1).

In order to confirm qualitatively the formation of the corresponding complexes in the ground state ^{1}H NMR titrations were carried out. Figure 5 shows very different trends in the case of receptor **8a** in the presence of Z-Trp-OH and Z-Ala-OH. In the former case, a clear upfield shift is observed for the different signals, suggesting the involvement of π interactions in the formation of the corresponding ground state complex. Such shifts are absent for the addition of Z-Ala-OH. Due to the weak interaction between pyrylium and substrate the corresponding association constant could not be calculated by ^{1}H NMR.

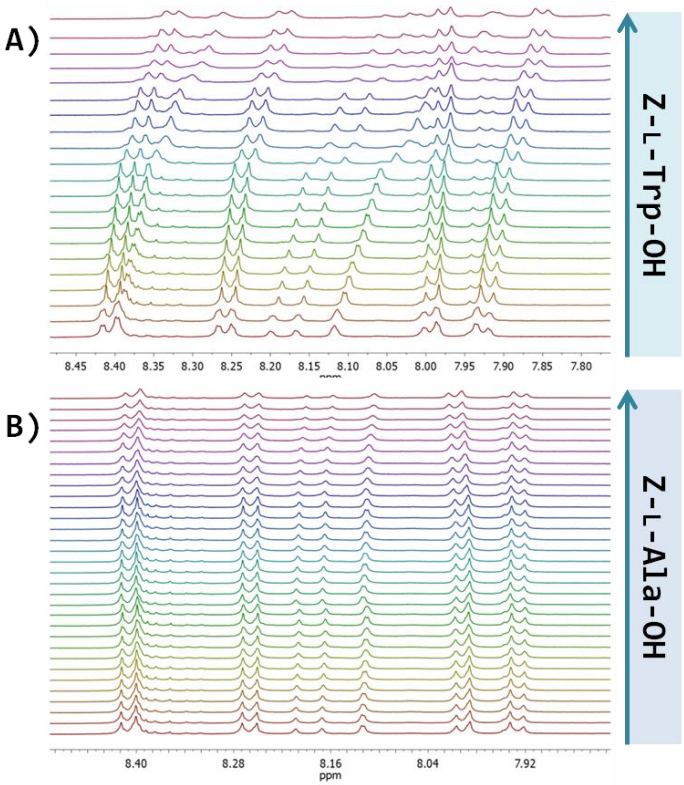


Figure 5. Stacked ^{1}H NMR spectra in CD_3CN of **8a** in the presence of increasing amounts of N-protected L-tryptophan (A) and L-alanine (0 to 45 mM).

Conclusions

In summary, we have prepared six new fluorescent pseudopeptidic pyrylium salts synthesized using valine or phenylalanine as building blocks and alkyl chains of different length. The new molecules have been characterized by ^{1}H / ^{13}C NMR, HRMS, UV-vis and Steady-State / Time-Resolved Fluorescence. The emissive properties (emission quantum yields, lifetimes and radiative deactivation constants) in different media have been described. The molecules here described show a single emission band, unlike related pyrylium compounds described in the literature. The interaction with amino acids in their N-protected forms has been studied by means of fluorescence quenching, using the Stern-Volmer methodology to determine static and dynamic constants. It has been found that the main quencher of the fluorescence of pyrylium salts **8a-c** and **9a-c** is Z-Trp-OH, and to a lesser extent Z-Tyr-OH and Z-Met-OH. No quenching was measured with Z-Ala-OH, Z-Val-OH and Z-Phe-OH. A detailed analysis of the fluorescence spectra using the Stern-Volmer formalism allows obtaining association constant between **8a** and

Z-Trp-OH (ca. 5 M^{-1}). Complementary ^{1}H-NMR titrations confirmed the existence of such weak ground state complex for Z-Trp-OH.

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