Synthesis of indole-containing diheteroarylethenes. New probes for photochromic FRET (pcFRET)

Luciana Giordano[1], Rudolf J. Vermeij[2], and Elizabeth A. Jares-Erijman[1,*]

[1.] Departamento de Química Orgánica. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Ciudad Universitaria Pabellón II Buenos Aires. Argentina. [2] Department of Molecular Biology, Max Planck Institute for Biophysical Chemistry, 37077 Göttingen, Germany Present address: MESA+ Institute for Nanotechnology, Faculty of Science and Technology, University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands.
Email: eli@go.fcen.uba.ar

Dedicated to Professor Rosa M. de Lederkremer

Abstract
This paper reports a synthesis of novel diheteroarylethenes functionalized for coupling to biomolecules starting from indole derivatives. The strategy is based on the derivatization at the N-atom in the indole substructure. TBDMS protection proved to be superior over BOC protection schemes, leading to higher yields in the overall synthesis. The suitability of the new derivatives as acceptors for pcFRET was calculated for selected donors.

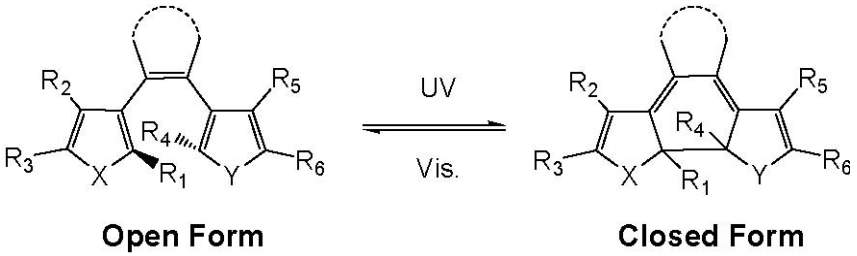
Keywords: Photochromism, FRET, diheteroarylethenes, synthesis, indole derivatives

Introduction

Photochromism is the result of reversible photoisomerization between two isomers that have distinct absorption spectra. The two isomers differ from one another not only in their absorption spectra but also in other physical and chemical properties.[1] Among the different photochromic compounds diheteroarylethenes are excellent candidates for optoelectronic devices due to their fatigue resistance and thermally irreversible conversion.[2]

Diheteroarylethenes undergo isomerization from an open to a closed form (scheme 1) upon irradiation with UV light. Visible light converts the closed form back into the original open form. Symmetric indole- or pyrrole containing diheteroarylethenes give rise to thermally unstable closed form isomers. By replacing one indole with a furane, thiophene or benzothiophene group, the asymmetric diheteroarylethene becomes thermally stable in the closed form. A number of methyl indole derivatives have been introduced by Irie and coworkers[3] and the resulting

photochromic compounds display interesting optical properties which makes them suitable as acceptors for energy transfer for a variety of fluorophore donors.



Scheme 1

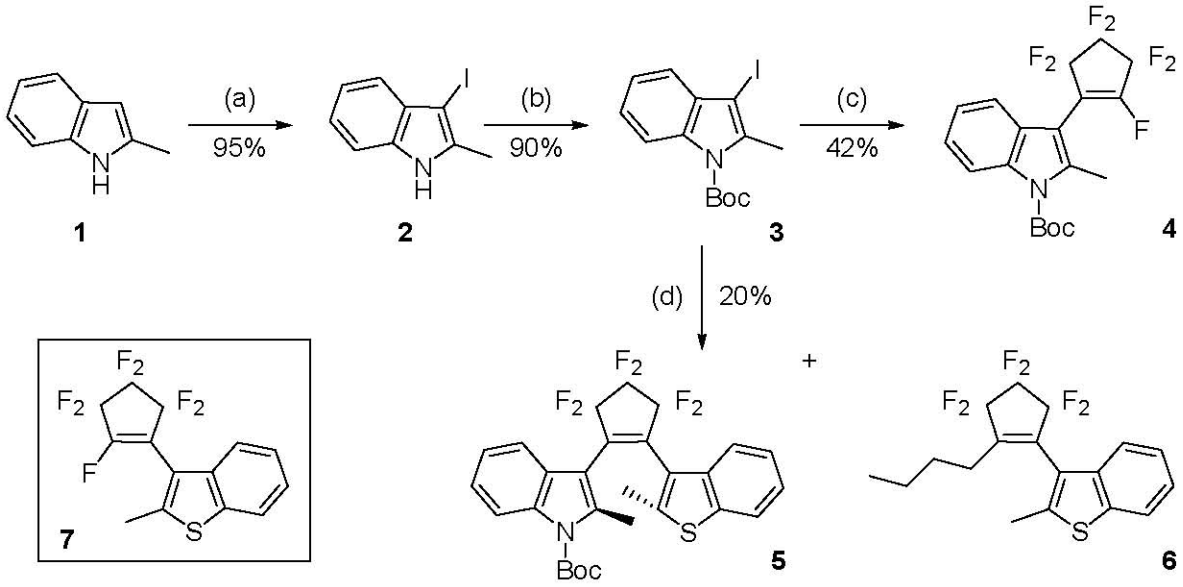
FRET (Förster Resonance Energy Transfer) is a physical process by which energy is transferred non-radiatively from an excited molecular fluorophore (donor) to another chromophore (acceptor) via long-range dipole-dipole coupling. The FRET acceptor need not be fluorescent, but must fulfill the requirements of having an absorption spectrum overlapping the emission spectrum of the donor with the respective transition moments in a favorable, i.e. non-orthogonal, relative orientation. We have shown previously that diheteroarylethenes can be used as acceptors for photochromic Förster Resonance Energy Transfer (pcFRET),[4, 5] a technique developed to perform the quantitative determination of FRET *in vivo*. The photochromic compound is converted from a colorless open form to a FRET-competent acceptor closed form upon irradiation with ultraviolet (UV) light. The open form lacks absorbance in the visible range. Thus, the overlap with the emission of the donor is negligible. The closed form has an absorption band overlapping the emission band of the donor, which can be switched back to the open, non-overlapping form by exposure to visible light. Multiple "on"/"off" FRET cycles can be generated by alternating exposure to UV and visible light.

Application of photochromic compounds as pcFRET acceptors in biology requires that they contain functional groups that can be used for their conjugation to (bio)molecules. Indole derivatives offer the opportunity for preparing a functionalized substituent on the N atom. Here we report the synthesis of asymmetric diheteroarylethenes bearing a removable protecting group at this position.

The diheteroarylhexafluorocyclopentenes are usually obtained by a reaction involving nucleophilic attack of a heteroaryllithium on octafluorocyclopentene, followed by elimination of fluoride groups.[2, 6] With indole as one of the heteroaryl functionalities, the heteroaryllitium species can be formed by halogen-lithium exchange reaction of the *N*-protected 3-haloindoles. Synthesis of these haloindoles is usually performed by electrophilic substitution. *N*-Protection of the indoles can be carried out before or after the halogenation step. As a result, the requirements of the protecting group are dictated by the lithium-halogen exchange reaction conditions. The synthesis and application of these *N*-protected indoles is well documented:[7, 8] alkyl, silyl, alkoxymethyl, acyl and other protecting groups have been used. Here we investigated their use for reaction with perfluorocyclopentene.

Results and Discussion

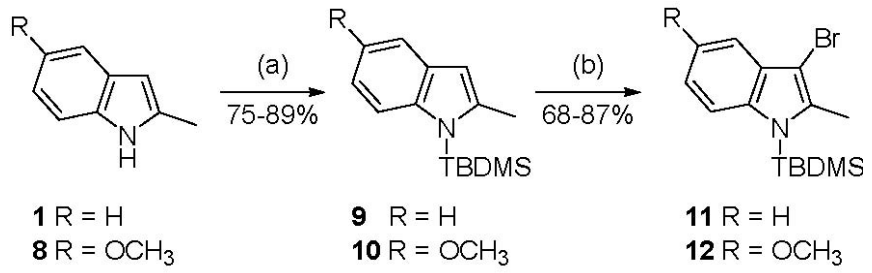
The first class of protective group investigated in this synthetic approach was the *t*-butoxycarbonyl group (BOC) (Scheme 2). 3-Iodo-2-methylindole **2** was obtained by iodination of 2-methylindole **1**[9, 10] which was followed by protection of the *N*-position of the indole nucleus to give **3**. In order to assess the best reaction conditions, different stoichiometric relationships between *n*-butyllithium and the alkylating reagent CH_3I were tested (reaction not shown in Scheme). The best yields (65-86%) were obtained when 2 equivalents of *n*-butyllithium and 1.5-3 equiv of CH_3I were used at -30 ºC. Lithiation of the 3-iodoindole **3**, followed by reaction with excess octafluorocyclopentene led to compound **4** in 42% yield. However, when a similar substitution reaction of the lithiated indole with compound **7** was attempted, the reaction proceeded sluggishly and only 29% of the desired product (**5**) was isolated, recovering 50% of starting material. When excess *n*-butyllithium was used in the halogen-metal exchange reaction, the desired product was isolated in 20% yield. In addition, the side product from reaction of unreacted *n*-butyllithium with **7** gave **6** in 48% (based on **7**).



a) KOH / I_2 / DMF b) Boc_2O / TEA / DMAP / CH_2Cl_2 c) i) *n*-BuLi (2.2 eq) / THF / -78 ºC ii) C_5F_8 d) i) *n*-BuLi (2.2 eq) / THF / -78 ºC ii) **7**

Scheme 2

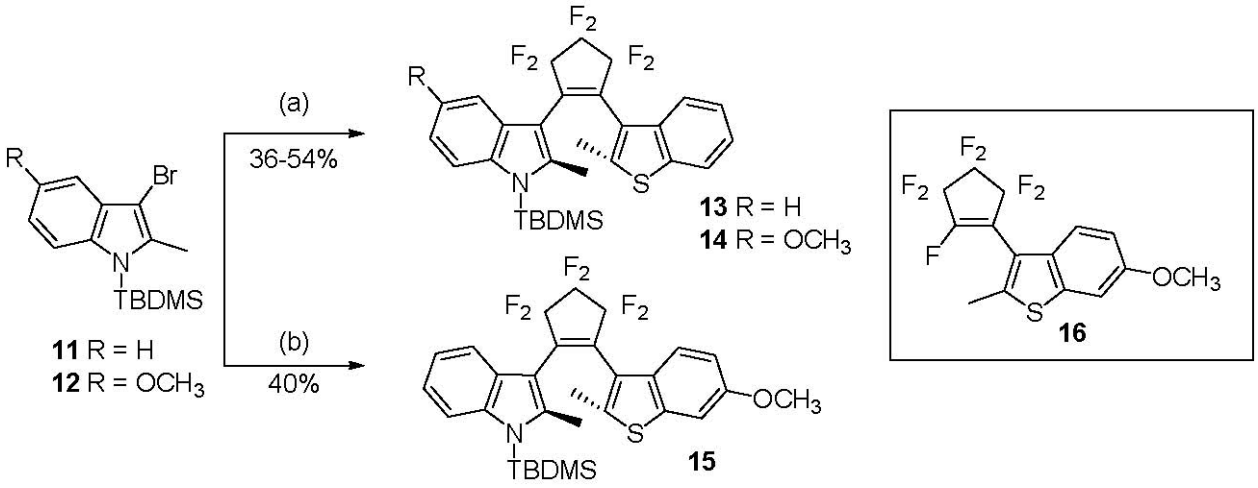
TBDMS was applied in an alternative protection strategy (Scheme 3). 2-Methylindole derivatives **1** and **8** were deprotonated with NaH and protected with TBDMSCl yielding **9** and **10**. Bromination with NBS at –78 ºC gave **11** and **12**.[11]



a) i) NaH / THF ii) TBDMSCl b) NBS / THF / -78 ºC

Scheme 3

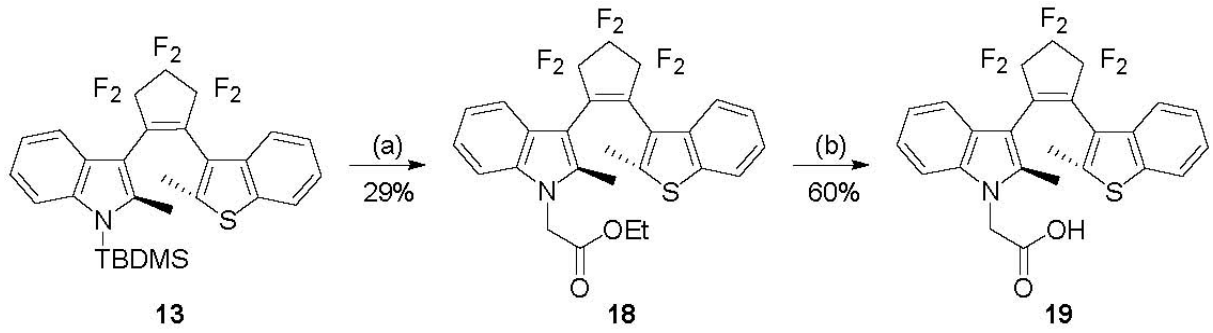
Lithiation of compounds **11** and **12** with *n*-butyllithium at –78 ºC, followed by reaction with **7** or **16** (**11** only) rendered the desired diheteroarylethenes **13** – **15** in reasonable yields (Scheme 4). These compounds already contain the photochromic moiety and bear orthogonally protected phenolic (OH; **14 only**) and indolic (NH) functionalities suitable for further derivatizations.



a) i) *n*-BuLi / THF / -78 ºC ii) **7** b) i) *n*-BuLi / THF / -78 ºC ii) **16**

Scheme 4

Deprotection of the indole-nitrogen proceeded smoothly with TBAF (Scheme 5). Immediate reaction with NaH followed by alkylation with ethyl bromoacetate in DMF rendered **18**, which by hydrolysis gave the final product **19**.[12, 13] Compound **19** contains a carboxylic acid group that can be further activated for conjugation with amine groups.



a) i) TBAF (1M) / THF ii) NaH /Ethyl bromoacetate / THF b) NaOH / THF

Scheme 5

Absorption properties. The absorption spectra of the open- and the closed forms are shown in Figure 1 **a** and **b**, respectively.

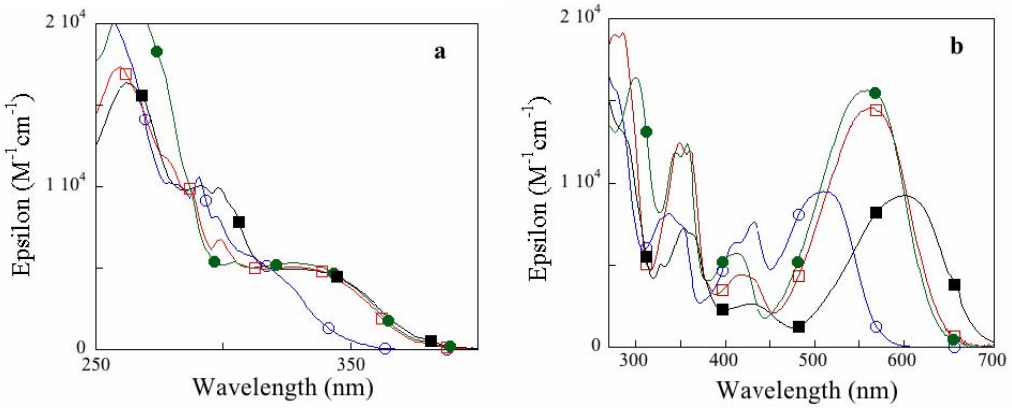


Figure 1. Absorption properties of compounds **5** (blue empty circles), **13** (red empty squares), **14** (black full squares), **15** (green full circles), in the open (**a**), and in the closed (**b**) form, respectively.

The optical properties of compounds **5**, **13-15** are given in Table I summarizing the absorption maxima corresponding to the open and closed forms, emission maxima of the open form, and the fractional conversion (to the closed form) achieved in the photostationary state by irradiation at the given wavelengths.

The compounds in the open form displayed absorption maxima at 247-270 nm (figure 1 **a**). Compounds **5**, **13**, **14** and **15** had a second maximum at 310-330 nm, and compound **14** presented an additional maximum at 338 nm. The open forms were fluorescent with emission maxima between 425 and 435 nm.

Table 1. Optical properties of compounds **5**, **13-15**

Compound	λ_{max} Open form nm; (ε, $M^{-1}cm^{-1}$)	Photo-conversion α (λ_{irr}, nm)	λ_{max} Closed form, nm; (ε, $M^{-1}cm^{-1}$)	λ_{max} Emission open form, nm
5	257 (20000) 292 (7900)	0.83 (300) 0.72 (320) 0.33 (340) 0.10 (360) 0.07 (380)	268 (16600) 338 (8200) 431 (7600) 511 (9500)	425
13	261 (17200) 331 (5000)	0.40 (300) 0.49 (320) 0.38 (340) 0.17 (360) 0.13 (380)	278 (19000) 300 (12000) 416 (4400) 560 (14600)	435
14	271 (14800) 300 (9800) 338 (4900)	0.69 (300) 0.72 (320) 0.64 (340) 0.38 (360) 0.23 (380)	353 (7200) 428 (2600) 600 (9200)	435
15	270 (20000) 333 (5500)	0.32 (300) 0.39 (320) 0.38 (340) 0.22 (360) 0.19 (380)	300 (16400) 358 (12400) 412 (5700) 558 (15600)	434

Photochromic properties. All synthesized diarylethenes underwent reversible photochromic reactions in cyclohexane upon alternating exposure to UV (340 nm) and visible (546 nm) light. Compounds **5**, **13** and **14** displayed two isosbestic points. Compound **5** at 299 and 316 nm; compound **13** at 313 and 321 nm, and compound **14** at 311 and 332 nm. On the other hand, compound **15** had one isosbestic point at 281 nm and compound **18** lacked an isosbestic point.

Upon irradiation with UV, the color of the solutions went from colorless to red (compounds **5**, **15**, and **16**) or blue (compound **14**). The effect of substituent groups on the *N* atom was evaluated by comparison of the optical properties of the BOC, TBDMS, CH_3,[2] and CH_2COOR derivatives. The closed form (Figure 1 **b**) displayed an absorption maximum at *ca*. 560 nm for the TBDMS, CH_3 and CH_2COOR substituents. The maximum was shifted to 511 nm for the less electron rich BOC group.

Introduction of a methoxy substituent is known to affect the absorption maxima and extinction coefficient values, depending on its position on the benzothiophene ring of a diarylethene.[14] A methoxy group on position 6 (compound **15**) exerted a negligible change in the

absorption maximum and displayed a slight increase in ε compared to **13**. On the other hand, the introduction of a methoxy group in the position 5 of the indole moiety (compound **14**) introduced a red-shift of *ca*. 40 nm compared to compound **13** and a decrease of ε to 9200 $M^{-1}cm^{-1}$.

Indole derivatives as acceptors for pcFRET. The modulation of the emission of a fluorescent donor by pcFRET is based on the difference in the absorption properties of the acceptor in its different photochromic forms. Compounds **5**, **13-15** were evaluated as switchable acceptors. Figure 2 displays the absorption spectra corresponding to the open and closed forms of compound **15** and the emission spectrum of Lucifer yellow (LYC). Compound **15** displays an effective spectral overlap between its absorption and the emission of the donor (LYC) only in the closed form.

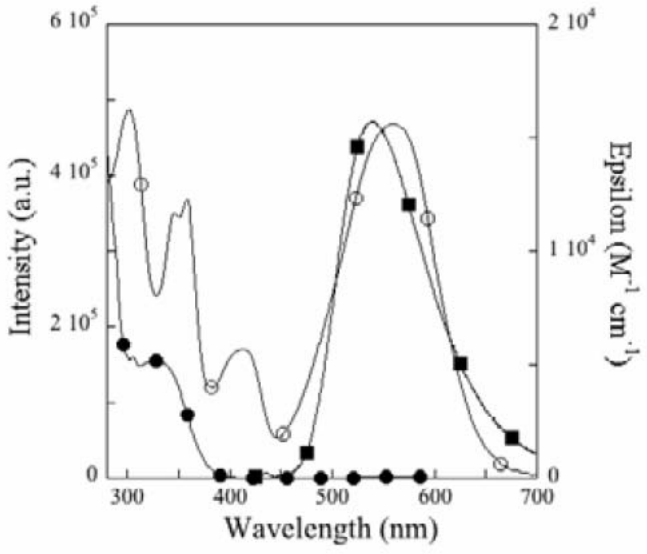


Figure 2. Spectral overlap of the open and closed forms of **15** and LYC. Open (full circles) and closed (empty circles) form extinction coefficients of **15** correspond to the right axis scale and the emission intensity of LYC (full squares) is given on the left axis.

The overlap integral *J* and the Förster distance R_0 were calculated for pairs consisting of the model donor, LYC and the photochromic acceptors, compounds **5**, **13-15** in their open and closed forms. Compound **14**, with a closed form displaced to the red, was additionally paired with donors sulforhodamine 101 and Nile red. In all cases, the two orders of magnitude difference in the overlap integral between the open and the closed forms demonstrated that the closed form was a competent acceptor, and the open form was not. The derived R_0 values were between 35-50 Å for the closed forms and decreased to 10-18 Å for the open forms.

Table 2. Overlap integral and Förster distances for donor - photochromic acceptor pairs in the open and closed form

Acceptor	Donor	*J* Open form, cm^6	R_0 Open form, Å	*J* Closed form, cm^6	R_0 Closed, Å
5	LYC	3.00 e-16	15	5.12 e-14	36
13	LYC	2.88e-17	10	9.82e-14	40
14	LYC	1.09 e-16	13	6.13 e-14	36
14	Sulforhodamine	1.94 e-16	18	1.06 e-13	51
14	Nile Red	1.82 e-16	17	9.06 e-14	48
15	LYC	1.88 e-16	14	9.63 e-14	40

Conclusions

The synthetic scheme using TBDMS as protective group was superior over the use of BOC for the preparation of functionalized, indole derived asymmetric diheteroarylethenes. The substitution on the indole ring at the *N*-atom had minor effects on the optical properties except for the BOC derivatives. Methoxy groups at position 5 on the indole moiety induced a red-shift of the absorption of the closed form to 600 nm and a decrease in ε. All photochromic compounds prepared in this work constitute good acceptors that can switch *on* and *off* the FRET process in pcFRET.

Experimental Section

General Procedures. Melting points were determined with a Fisher Jones apparatus and are uncorrected. NMR measurements were carried out on a Bruker 200 MHz AM, 400 MHz, 500 MHz AMX, Mercury 300 MHz (Varian) or on a INOVA 500 MHz (Varian) NMR spectrometer. Chemical shifts are in ppm (internal TMS) and coupling constants in Hz. All reactions were monitored by thin-layer chromatography on Merck silica gel plates (60F-254). Column chromatography was performed on silica gel (Merck, 230-400 mesh ASTM). The purities of all final compounds were checked HPLC analysis on a Waters system fitted with a MZ-Semipreparative Kromasil 100 Sil 5 μm silica column (25 cm long, 10 mm internal diameter). ESI and HRMS were measured on an APEX IV 7 Tesla-Fourier Transform Ion Cyclotron Resonance (FTICR)- Mass spectrometer (Bruker) or on a TSQ 7000 Triple-Stage-Quadrupol-Instrument (Finnigan) with Electrospray-Ionisation. Elementary analyses were measured with a Leco CHN2000 analyser with burn unit MICRO U/D (Heraeus). Absorption and fluorescence spectra were measured with a UVIKON 943 Double Beam UV/Vis absorption spectrophotometer and a Perkin Elmer LS50B fluorescence spectrophotometer, respectively. Photoirradiation was carried out using a Superlite SUV-DC-P system incorporating a 200W DC Super-Pressure short arc lamp coupled to a light guide for high UV transmission and an

electronic timer for exposure time control (Lumatec GmbH, Munich, Germany). Monochromic light was obtained by passing the light a band-pass filter ($\Delta\lambda_{1/2}$=10 nm). The photoconversions were determined with an Ocean Optics fiber optics spectrometer system with a cuvette holder specially modified to allow simultaneous photoconversion and spectral monitoring. Excitation for absorption and fluorescence was with a DT1000A deuterium/tungsten lamp, and detection was with a SD2000 series dual fiber optics spectrometer optimized for detection in the 250-800 nm spectral region.

FRET Methods. , the critical Förster distance for 50% FRET efficiency, is defined by $R_o^6 = 8.785 \cdot 10^{-5} \kappa^2 \Phi_D J n^{-4}$ units, nm^6) where Φ^D is the quantum yield of the donor in the absence of acceptor, n is the refractive index of the medium, κ^2 is the orientation factor between donor and acceptor (here assumed to be 2/3, the value corresponding to rapid and isotropic reorientation of donor and acceptor during the excited state), and J is the spectral overlap integral between donor and acceptor, given by $J = \int F_\lambda^D \varepsilon_\lambda^A \lambda^4 d\lambda$, where F_λ is the normalized donor fluorescence spectrum and ε_λ is the wavelength-dependent molar extinction coefficient (M^{-1} cm^{-1}) of the acceptor. For an isolated donor-acceptor pair, the FRET efficiency E varies according to $E = [1 + (r_{DA}/R_o)^6]^{-1}$.

Materials. 2-methyl-*1H*-indole (**1**), 5-methoxy-2-methyl-*1H*-indole (**8**), are commercially available and were obtained from Aldrich Chem. Co. Compounds **7**[15] and **16**[14] were prepared as previously described.

3-Iodo-2-methyl-1*H*-indole (2). A solution of I_2 (9.7 g, 38 mmol) in DMF (65 ml) was added dropwise to a solution of **1** (5.0 g, 38 mmol) and KOH (5.3 g, 95 mmol) in DMF (80 ml) at room temperature and stirred for 30 min. The reaction mixture was poured into ice and water (1 l) containing ammonia (0.5 %) and sodium metabisulphite (0.1 %). The orange-white precipitate was filtered and washed with cold water. The obtained 3-iodo-2-methyl-1*H*-indole **2** (9.3 g, 36 mmol, 95%) was used without further purification. M.p: 82 ºC (lit:[16] 83-84 ºC). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.16 (1H, bs, NH), 7.35 (1H, m), 7.25 (1H, m), 7.16 (2H, m), 2.48 (3H, s).

***tert*-Butyl 3-iodo-2-methyl-1*H*-indole-1-carboxylate (3).** Compound **2** (1.57 g, 6.1 mmol) was dissolved in dichloromethane (150 ml) and treated with di-*tert*-butyldicarbonate (1.57 g, 7.2 mmol), triethylamine (2.7 ml, 19 mmol), DMAP (80 mg, 0.66 mmol) and the reaction mixture was stirred at room temperature for 30 min. The solution was washed twice with sodium metabisulphite (5%, 50 ml each time), dried over Na_2SO_4 and concentrated under reduced pressure. Chromatography over silica gel (hexane:ethyl acetate = 8:2) gave **3** (1.99 g, 5.6 mmol, 90%) as a colorless solid. M.p 55-56 º C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.06 (1H, dd, J = 7.1 and 2.2 Hz), 7.36 (1H, dd, J = 6.5 and 2.7 Hz), 7.27-7.30 (2H, m), 2.72 (3H, s), 1.69 (9H, s). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 149.8, 138.2, 136.0, 131.2, 124.5, 123.2, 120.9, 115.4, 84.3, 71.4, 28.2 (3C), 17.9. Anal. Calcd. for $C_{14}H_{16}INO_2$ (357.19) C 47.08, H 4.52, N 3.92. Found: C 47.02,

H 4.41, N 3.85. MS (ESI): m/z (%): 357 ($M^{+\cdot}$, 43); 301 (100); 257 (80); 130 (15), 57 (97).

3-(2,3,3,4,4,5,5-Heptafluorocyclopent-1-enyl)-1-(*t*-butoxycarbonyl)-2-methyl-1*H*-indole (4). A solution of *n*-BuLi in hexane (1.6 M, 1.0 ml, 1.6 mmol) was added to a stirred solution of **3** (0.50 g, 1.4 mmol) in anhydrous THF (6 ml) at -20 ºC under argon atmosphere. After the addition was complete, the resulting mixture was cooled to -78 ºC and octafluorocyclopentene (0.19 ml, 1.4 mmol) was added in a single portion. The mixture was stirred for 1 h at -78 º C. The mixture was allowed to warm to room temperature and hydrolyzed with an aqueous HCl (1 N, 7 ml) solution. The mixture was extracted with ethyl acetate (4 x 5 ml). The combined organic layers were washed with H_2O and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica with hexane and followed by HPLC (silica gel column, hexane) to give **4** as a white solid (250 mg, 0.59 mmol, 42 %). M.p.: 74-75 ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.14 (1H, d, *J* = 8.3 Hz), 7.42 (1H, d, *J* = 7.3 Hz), 7.27-7.35 (2H, m), 2.55 (3H, s), 1.71 (9H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 149.9, 139.3, 135.9, 127.0, 124.6, 123.6, 118.7, 115.6, 85.1, 28.2 (3C), 15.2. (Resonances of the *C*-atoms of the fluorinated cyclopentene moiety could not be observed due to low intensity and extensive splitting). Anal. Calcd. for $C_{19}H_{16}F_7NO_2$ (423.33): C 53.91; H 3.81. Found: C 53.70; H 3.66. HRMS: Calcd. 423.1069. Found: 423.1069. MS (ESI) m/z (%): 423 ($M^{+\cdot}$, 22), 367 (48), 323 (66), 301 (24), 257 (22), 57 (100).

3-[3,3,4,4,5,5-Hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-1-(*t*-butoxycarbonyl)-2-methyl-1*H*-indole (5). A solution of *n*-BuLi in hexane (1.6 M, 0.77 ml, 1.2 mmol) was added to a stirred solution of **3** (200 mg, 0.56 mmol) in anhydrous THF (3 ml) at -78 ºC under nitrogen atmosphere. The resulting mixture was stirred for 30 min at -78 ºC and a solution of **7** (200 mg, 0.61 mmol) in anhydrous THF (1 ml) was added. After the addition was complete, the mixture was allowed to return to room temperature and it was hydrolyzed with an aqueous HCl (1N, 5 ml) solution. The mixture was extracted with ethyl acetate (3 x 5 ml). The resulting organic phase was washed with H_2O and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica with hexane/ethyl acetate 98:2 to give **5** as a slightly yellow solid (70 mg, 0.13 mmol, 20 %). M.p.: 71-72 ºC. ^{1}H-NMR (300 MHz, $CDCl_3$) (323 K): δ 8.01 (1H, *d, J* = 7.5 Hz), 7.67 (1H, d, *J* = 7.0 Hz), 7.60 (1H, d, *J* = 6.5 Hz), 7.50 (1H, d, *J* = 7.4 Hz), 7.17-7.27 (4H, m), 2.36-2.31 (6H, bs). Anal. calcd. for $C_{28}H_{23}F_6NO_2S$ (551.55): C 60.97, H 4.20, N 2.54. Found: C 60.74, H 3.96, N 2.62. HRMS Calcd.: 551.1354; found: 551.1354. MS (EI) m/z (%): 551 ($M^{+\cdot}$, 13), 495 (22), 451 (14), 57 (100).

3-(2-Butyl-3,3,4,4,5,5-hexafluorocyclopent-1-enyl)-2-methylbenzo[*b*]thiophene (6). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.78 (1H, d, *J* = 7.8 Hz), 7.49 (1H, d, *J* = 7.9 Hz), 7.39 (1H, m), 7.34 (1H, m), 3.81 (1H, m), 3.65 (1H, m), 2.51 (3H, s), 1.46 (2H, m), 1.18 (2H, m), 0.73 (3H, t, *J* = 8 Hz).

1-(*tert*-Butyldimethylsilanyl)-2-methyl-1*H*-indole (9). Sodium hydride (350 mg, 9.2 mmol, mineral oil suspension 50%) was added in portions to a stirred solution of **1** (1.0 g, 7.6 mmol) in anhydrous THF (15 ml) and the mixture was stirred for 10 min. *tert*-Butyl(chloro)dimethylsilane (1.4 g, 9.2 mmol) was added and the mixture was stirred for 12 h at room temperature under

nitrogen atmosphere. The reaction was quenched with water (10 ml). The mixture was extracted with ethyl acetate (2 x 5ml). The organic layer was washed with saturated aqueous NaCl solution (10 ml), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica (cyclohexane) to give **9** as a colorless oil (1.67 g, 6.8 mmol, 89%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.46-7.53 (2H, m), 7.03-7.08 (2H, m), 6.34 (1H, s), 2.49 (3H, s), 0.97 (9H, s), 0.67 (6H, s). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 142.7, 142.0, 131.3, 120.3, 119.6, 119.1, 114.1, 106.1, 26.8 (3C), 20.6, 17.5, -0.5 (2C).

3-Bromo-1-(*tert*-butyldimethylsilanyl)-2-methyl-1*H*-indole (11). *N*-Bromosuccinimide (145 mg, 0.81 mmol) was added to a solution of **9** (200 mg, 0.81 mmol) in anhydrous THF (5 ml) at -78 º C. After 2 h, the mixture was allowed to warm up to room temperature. The mixture was evaporated under reduced pressure. The residue was purified by flash column chromatography (silica, cyclohexane) to give **11** as a white solid (180 mg, 0.56 mmol, 68%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.48-7.52 (2H, m), 7.18-7.14 (2H, m) 2.53 (3H, s), 1.00 (9H, s), 0.69 (6H, s). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 140.9, 138.4, 130.0, 121.6, 120.4, 118.4, 114.3, 95.9, 26.7 (3C), 20.5,15.5, -0.3 (2C).

1-(*tert*-Butyldimethylsilanyl)-3-[3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-1*H*-indole (13). A solution of *n*-BuLi in hexane (1.6 M, 0.53 ml, 0.61 mmol) was added to a stirred solution of **11** (180 mg, 0.56 mmol) in anhydrous THF (3 ml) at -78 ºC under argon atmosphere. The resulting orange solution was stirred for 30 min at -78 ºC and a solution of **7** (210 mg, 0.61 mmol) in anhydrous THF (1 ml) was added. After 30 min at -78 ºC, the mixture was allowed to warm to room temperature and it was hydrolyzed with an aqueous HCl (1N, 5 ml) solution. The mixture was extracted with ethyl acetate (2 x 5 ml). The combined organic layers were washed with H_2O and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica with hexane/ethyl acetate 98:2 to give a slightly yellow solid (125 mg, 0.22 mmol 40 %). Purification by HPLC (silica 60, hexane) gave **13** as a white solid (115 mg, 0.2 mmol, 37%). M.p.: 162-163 ºC. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43-7.64 (4H, m), 7.06-7.24 (4H, m) 2.27 (3H, bs), 1.98 (3H, s), 0.60 (9H, s), 0.54 (6H, s). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 142.8, 142.2, 141.8, 138.0, 129.0, 124.3, 124.1, 122.2, 121.7, 121.5, 120.7, 120.6, 119.1, 119.0, 118.9, 114.2, 26.3 (3C), 20.5, 15.8, 15.1, -0.2 (2C). (Resonances of the *C*-atoms of the fluorinated cyclopentene moiety could not be observed due to low intensity and extensive splitting). Anal. Calcd. for $C_{29}H_{29}F_6NSSi$ (565.69): C 61.57, H 5.17; found: C 61.51, H 4.95. MS (ESI) m/z (%): 565 ($M^{+\cdot}$, 68), 509 (22), 73 (100). HRMS. Calcd.: 565.1694. Found: 565.1694.

1-(*tert*-Butyldimethylsilanyl)-3-[3,3,4,4,5,5-hexafluoro-2-(6-methoxy-2-methyl-benzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-1*H*-indole (15). A solution of *n*-BuLi in hexane (1.6 M, 0.77 ml, 0.88 mmol) was added to a stirred solution of **11** (260 mg, 0.80 mmol) in anhydrous THF (3 ml) at -30 ºC under argon atmosphere. The resulting mixture was stirred for 30 min at -30 ºC and the mixture was cooled at – 78 º C. A solution of **16** (300 mg, 0.80 mmol) in anhydrous THF (1 ml) was added. After 30 min at -78 ºC, the mixture was allowed to warm to room temperature and it was hydrolyzed with an aqueous HCl (1N, 5 ml) solution. The mixture

was extracted with ethyl acetate (2 x 5 ml). The combined organic layers were washed with H_2O and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica with hexane/ethyl acetate 98:2 to give a white solid (180 mg, 0.30 mmol, 38 %). Purification by HPLC (silica 60, hexane) gave **15** as a white solid (170 mg, 0.29 mmol, 36 %). M.p.: 152-153 ºC. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.61 (1H, d, J = 8.1 Hz), 7.44 (1H, d, J = 8.3 Hz), 7.06-7.13 (4H, m), 6.81 (1H, bs), 3.80 (3H, s), 2.19 (3H, bs), 1.97 (3H, s), 0.63 (9H, s), 0.55 (6H, s). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.1, 142.8, 141.8, 139.4, 132.1, 129.0, 122.9, 121.5, 120.8, 119.1, 119.0, 118.9, 114.2, 114.0, 105.7, 104.6, 55.5, 26.4 (3C), 20.5, 15.8, 15.0, -0.1 (2C). (Resonances of the *C*-atoms of the fluorinated cyclopentene moiety could not be observed due to low intensity and extensive splitting). Anal calcd for $C_{30}H_{31}F_6NOSSi$ (595.71): C 60.49, H 5.25. Found: C 60.64, H 5.00. MS (EI) m/z (%): 565 ($M^{+\cdot}$, 100), 539 (25), 538 (26), 73 (76). HRMS. Calcd: 595.1800. Found: 595.1800.

1-(*tert*-Butyldimethylsilanyl)-2-methyl-5-methoxy-1*H*-indole (10). Sodium hydride (130 mg, 3.7 mmol, mineral oil suspension 50%) was added in portions to a stirred solution of **8** (0.50 g, 3.1 mmol) in anhydrous THF (10 ml) and the mixture was stirred for 10 min. *tert*-Butyl(chloro)dimethylsilane (0.56 g, 3.7 mmol) was added and the mixture was stirred for 12 h at room temperature under nitrogen atmosphere. The reaction was quenched with water (10 ml). The mixture was extracted with ethyl acetate (2 x 5ml). The combined organic layers were washed with saturated aqueous NaCl (10 ml) solution, dried over Na_2SO_4, filtered and concentrated under pressure. The residue was purified by flash column chromatography on silica (cyclohexane) to give **10** as colorless oil (635 mg, 2.3 mmol, 74%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.37 (1H, d, J = 9 Hz), 6.95 (1H, d, J = 2.4 Hz), 6.70 (1H, dd, J = 9 and 2.4 Hz) 6.26 (1H, s), 3.83 (3H, s), 2.46 (3H, s), 0.95 (9H, s), 0.64 (6H, s).

3-Bromo-1-(*tert*-butyldimethylsilanyl)-2-methyl-5-methoxy-1*H*-indole (12). *N*-Bromosuccinimide (400 mg, 2.3 mmol) was added to a solution of **10** (635 mg, 2.3 mmol) in anhydrous THF (10 ml) at -78 º C. After 2 h, the mixture was allowed to warm up to room temperature. The mixture was concentrated under reduced pressure. The crude residue was purified by flash column chromatography (silica, cyclohexane) to give **12** as a white solid (710 mg, 2.0 mmol, 87%). ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.36 (1H, d, *J* = 9.0 Hz), 6.91 (1H, d, *J* = 2.4 Hz), 6.76 (1H, dd, *J* = 9.0 and 2.4 Hz), 3.88 (3H, s), 2.49 (3H, s), 0.97 (9H, s), 0.65 (6H, s) ^{13}C-NMR (50 MHz, $CDCl_3$): δ 154.6, 139.1, 135.6, 130.6, 115.1, 111.4, 100.1, 95.5, 55.7, 26.7 (3C), 20.4, 15.5, -0.4 (2C).

1-(*tert*-Butyldimethylsilanyl)-3-[3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-5-methoxy-1*H*-indole (14). A solution of *n*-BuLi in hexane (1.0 M, 0.93 ml, 0.93 mmol) was added to a stirred solution of **12** (300 mg, 0.85 mmol) in anhydrous THF (10 ml) at -30 ºC under argon atmosphere. The resulting orange mixture was stirred for 30 min at -30 ºC and the reaction was cooled at -78 ºC. A solution of **7** (290 mg, 0.85 mmol) in anhydrous THF (2 ml) was added. After 30 min at -78 ºC, the mixture was allowed to warm up to room temperature and it was hydrolyzed with an aqueous HCl (1N, 5 ml) solution. The

mixture was extracted with ethyl acetate (2 x 5 ml). The combined organic layers were washed with H_2O and saturated aqueous NaCl solution, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography on silica with cyclohexane/ethyl acetate 98:2 to give **14** as a yellow solid (270 mg, 0.45 mmol 53 %). M.p.: 167-168 ºC. ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.65 (1H, bs), 7.22-7.31 (4H, m), 7.01 (1H, bs) 6.71 (1H, d, *J* = 8.5 Hz), 3.76 (3H, s), 2.24 (3H, bs), 1.98 (3H, bs), 0.61 (9H, s), 0.53 (6H, s). ^{13}C-NMR (125 MHz, $CDCl_3$): δ 154.6, 143.5, 142.3, 138.2, 136.7, 129.7, 124.4, 124.2, 122.4, 122.3, 121.8, 114.9, 111.1, 101.1, 55.7, 26.4 (3C), 20.5, 16.0, -0.1 (2C). (Resonances of the *C*-atoms of the fluorinated cyclopentene moiety could not be observed due to low intensity and extensive splitting). MS (EI) m/z (%): 596 ($M+H^+$, 10), 480 (100).

3-[3,3,4,4,5,5-Hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-1*H*-indole (17). A solution of tetrabutylammonium fluoride (TBAF) in THF (1M, 0.11 ml, 0.11 mmol) was added to a stirred solution of **13** (60 mg, 0.11 mmol) in THF (1 ml), under argon atmosphere. After the solution was stirred for 20 min at room temperature, it was poured into a saturated solution of Na_2CO_3 (2 ml) and extracted with CH_2Cl_2 (3 x 2 ml). The organic layers were combined and washed with H_2O (2 ml), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to flash column chromatography on silica gel (cyclohexane/ethyl acetate 8:2) to give 40 mg (0.09 mmol, 84%) of the desired compound. Compound **17** was used immediately in the next reaction. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.97 (1H, bs), 7.59-7.69 (3H, m), 7.23-7.28 (2H, m), 7.07-7.18 (3H, m), 2.24 (3H, s), 1.94 (3H, s).

Ethyl {3-[3,3,4,4,5,5-hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-1*H*-indol-1-yl}acetate (18). NaH (2mg, 0.06 mmol, mineral oil suspension, 50%) was added to a stirred solution of **17** (20 mg, 0.04 mmol) in DMF (0.5 ml) under N_2. After stirring for 30 min, the mixture was cooled and one drop of ethyl bromoacetate was added. The resulting red-brown solution was stirred for 2.5 h and diluted with 5 ml of ethyl acetate. The organic layer was washed with H_2O (3 x 3 ml) and saturated NaCl solution, dried over N_2SO_4 and concentrated to give red oil. The oil was purified by flash column chromatography on silica (cyclohexane/ethyl acetate 8:2) to give **18** (8 mg, 0.01 mmol, 34%) as a slightly yellow solid. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.62-7.69 (3H, m), 7.25-7.31 (3H, m), 7.10-7.15 (2H, m), 4.63 (2H, s), 4.06 (2H, q, *J* = 7.1 Hz), 2.19 (3H, s), 1.90 (3H, s), 1.09 (3H, t, *J* = 7.1 Hz).

{3-[3,3,4,4,5,5-Hexafluoro-2-(2-methylbenzo[*b*]thien-3-yl)-cyclopent-1-enyl]-2-methyl-1*H*-indol-1-yl}acetic acid (19). Compound **18** was dissolved in THF (1 ml), an aqueous solution of NaOH (1M, 0.5 ml, 0.5 mmol) was added, and the mixture was stirred under reflux for 1 h. The reaction mixture was cooled to room temperature and diluted with H_2O. After acidification of the solution to pH 1 using concentrated HCl the suspension was extracted with CH_2Cl_2 (2 x 2 ml) and the organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The product was purified by preparative thin layer chromatography on reverse phase silica (RP18) with ethyl acetate/2-propanol/H_2O 4:3:2 as a solvent to give **19** (4 mg, 0.008 mmol, 60%) as a slightly yellow solid**.** ^{1}H-NMR (200 MHz, $CDCl_3$): δ 7.62-7.66 (3H, m), 7.26 (3H, m), 7.13-7.15 (2H, m), 4.65 (2H, s), 2.18 (3H, s), 1.88 (3H, s).

Acknowledgements

The authors would like to thank Dr. Leonardo Erijman and Dr. Thomas Jovin for careful reading of the manuscript. E.A.J.-E. is indebted to the Agencia Nacional de Promoción de la Ciencia y Tecnología (ANPCyT), Fundación Antorchas, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Secretaría de Ciencia, Tecnología e Innovación Productiva (SECyT), Germany-Argentine DLR-BMBF-SECyT, and the Universidad de Buenos Aires (UBA) for financial support. E.A.J.-E. is recipient of the grant I/77 897 from the Volkswagen Foundation for collaborative work on pcFRET with Dr. Thomas Jovin (MPIBPC-Goettingen, Germany).

References

1. Hirshberg, *Compt. Rend.* **1950**, *231*, 903.
2. Irie, M. *Chem. Rev.* **2000**, *100*, 1685.
3. Yagi, K.; Irie, M. *Bull. Chem. Soc. Jpn.* **2003**, *76*, 1625.
4. Giordano, L.; Jovin, T. M.; Irie, M.; Jares-Erijman, E. A. *J. Am. Chem. Soc.* **2002**, *124*, 7481.
5. Jares-Erijman, E. A.; Jovin, T. M. *Nat. Biotechnol.* **2003**, *21*, 1387.
6. Hanazawa, M.; Sumiya, R.; Horikawa, Y.; Irie, M. *J. Chem. Soc., Chem. Commun.* **1992**, 206.
7. Greene, T. W.; Wuts, P. G. M. *Protective Goups in Organic Synthesis*. Wiley-Interscience: New York, 3rd Edn, 1999.
8. Dhanak, D.; Reese, C. B. *J. Chem. Soc., Perkin Trans. 1* **1986**, 2181.
9. Witulski, B.; Buschmann, N.; Bergstrasser, U. *Tetrahedron* **2000**, *56*, 8473.
10. Bodwell, G. J.; Li, J. *Org. Lett.* **2002**, *4*, 127.
11. Amat, M.; Hadida, S.; Sathyanarayana, S.; Bosch, J. *J. Org. Chem.* **1994**, *59*, 10.
12. Cross, P. E.; Dickinson, R. P.; Parry, M. J.; Randall, M. J. *J. Med. Chem.* **1986**, *29*, 1637.
13. Bell, M. R.; Dambra, T. E.; Kumar, V.; Eissenstat, M. A.; Herrmann, J. L.; Wetzel, J. R.; Rosi, D.; Philion, R. E.; Daum, S. J.; Hlasta, D. J.; Kullnig, R. K.; Ackerman, J. H.; Haubrich, D. R.; Luttinger, D. A.; Baizman, E. R.; Miller, M. S.; Ward, S. J. *J. Med. Chem.* **1991**, *34*, 1099.
14. Frigoli, M.; Mehl, G. H. *Chem.- Eur. J.* **2004**, *10*, 5243.
15. Nakashima, H.; Irie, M. *Polymer J.* **1998**, *30*, 985.
16. Orazi, O.; Corral, R. A.; Bertorello, H. E. *J. Org. Chem.* **1965**, *30*, 1101.