An Expedient Synthesis of Cationic Rhodamine Fluorescent Probes Suitable for Conjugation to Amino Acids and Peptides

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Abstract: Rhodamine 19 benzyloxycarbonylmethyl ester bromide **8** and rhodamine 19 4-chloromethyl-1-phenylmethyl ester chloride **12** act as precursors to cationic fluorescent probes. Molecules containing free amine or carboxylate functional groups, respectively, can be attached in a one-step procedure, yielding the desired probes without the need for chromatographic purification. As a proof of concept the method was applied to the attachment of amino acid and dipeptide residues through either the *N*- or *C*-termini. The precursor molecules **8** and **12** are readily synthesized from the inexpensive, commercially available dye rhodamine 6G.

Key words: fluorescent probes, rhodamines, amino acid derivatives, peptides

The application of fluorescent molecular probes in high-throughput assays, cellular imaging and for single molecule detection is of growing importance,[1] particularly with the advent of fluorescent resonance energy transfer, fluorescence correlation spectroscopy and fluorescence polarization techniques. Of the various types of fluorescent dyes that are routinely used, the rhodamine family is a particularly important class of long-wavelength visible dye. The use of fluorescent rhodamine containing molecules as probes is of importance in several different fields, including molecular biology and medicine, combinatorial chemistry, polymer chemistry, chromatography, as well as for single molecule detection in cells.[2–5] Conjugation of biologically relevant molecules to rhodamine probes typically occurs through reaction of a nucleophilic functional group on the molecule of interest with an activated ester, sulfonyl chloride or isothiocyanate functionality present on the lower ring of the rhodamine dye **1**. Although several such rhodamine probe molecules and their precursors are commercially available, many of these are quite expensive. We now report an alternative attachment strategy for the formation of cationic dyes **3**, using inexpensive rhodamine 6G **2** as a precursor (Figure 1).

We envisaged that commercially available rhodamine 6G **2** would serve as a useful precursor for fluorescent probes after hydrolysis of the ethyl ester. Lactone **4** was prepared by pyrolysis of **2** using a reported procedure (Scheme 1).[6]

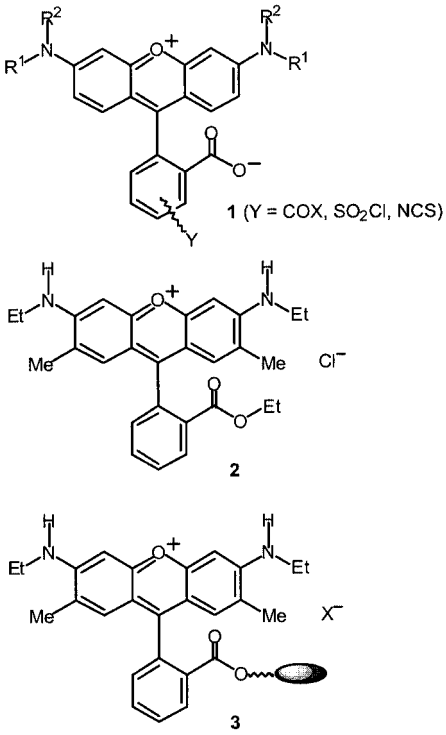

Figure 1

The pyrolysis can be achieved on up to 20 g scale by heating **2** for 4–5 hours at 265–275 °C under vacuum. Initially, conjugation to the nitrogen atoms of **4** was attempted through amide or carbamate linkages. Such molecules could potentially be used as mechanism-based probes, only displaying high fluorescence on amide/carbamate bond cleavage. Several attempts were performed for functionalization of the amino group on the rhodamine ring system, but clean conversions were generally not observed, and only the *N*-acetyl derivative **5** was prepared cleanly in low yield. Once protected the second free amine group of **5** it is more reactive, and functionalization as the *N*-CBz derivative **6**, for instance, was achieved in high yield. A much cleaner system for carbamate or amide attachment (e.g., for the C-terminal attachment of simple peptides through an amide linkage) utilizes the more reactive, and also more expensive, rhodamine 110 as a precursor (which contains free NH_2 rather than NHEt groups on the rhodamine ring).[7] Given the poor reactivity profile of

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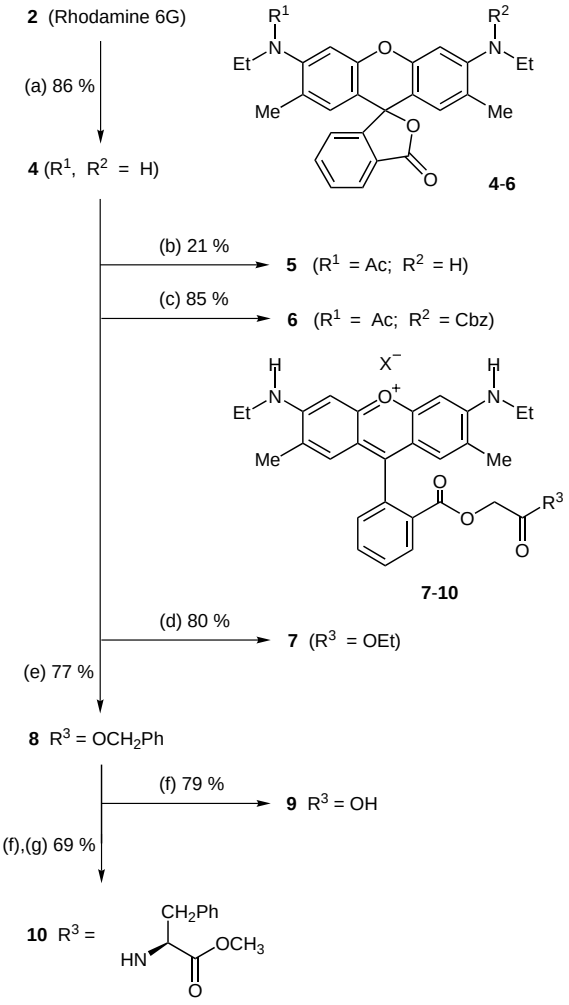
4 shown in these exploratory studies, we next turned our attention to conjugation through the carboxylate residue of rhodamines **1** (Y = H).

The formation of cationic probe molecules **3** can in principle be achieved through the conjugation of biologically relevant molecules to rhodamine dyes through the 2-carboxylate group in **1** (Y = H). Rhodamine 6G **2** constitutes a trivial example of such a probe, and it is used for instance as a cationic cell permeant lipophilic probe.[8] Reaction of **4** with activated alkyl halides (α-halo esters or benzyl halides), *i*-Pr_2NEt and a catalytic quantity of sodium iodide in refluxing acetonitrile, resulted in lactone ring-opening and alkylation to form esters **7**, **8**, **11** and **12** in high yields (Schemes 1 and 2).[9] The preparation of these compounds could also be accomplished, albeit in lower yield, through initial hydroxide induced hydrolysis of **2** followed by direct alkylation. For example, reaction of **2** with LiOH in THF–H_2O and alkylation with ethyl 2-bromoacetate under the above conditions afforded **7** in 36% overall yield. Hydrogenolysis of benzylic ester **8** afforded the carboxylic acid derivative **9**, which serves as a useful precursor for conjugation of molecules containing amine, or potentially thiol, functionality. For example, DCC/*N*-hydroxysuccinimide promoted amide bond formation between **9** and L-phenylalanine methyl ester gave derivative **10**.[10] Such a strategy could be further extended for the conjugation of more complex amines or peptides.[11] An alternative recent report by Adamczyk outlines a method for the direct attachment of reactive primary amines through their reaction with **2** using Hünig's base in DMF.[12] There are also some reports in the patent literature in which compounds of type **1** (Y = H) are directly conjugated to amines at the carboxylate group to form amides.[13]

For the attachment of the carboxylic acid group of peptide derivatives and other compounds to rhodamines an alternative strategy is required. We envisaged that a benzyl chloride functionalized rhodamine derivative would serve as an excellent electrophile for the alkylation of carboxylic acids. Such a compound could be formed by the 1:1 coupling of **4** with a *bis*-benzylic halide. Preliminary experiments using *i*-Pr_2NEt and a catalytic quantity of sodium iodide in refluxing acetonitrile, using benzylbromide as a model electrophile afforded benzyl ester **11** (Scheme 2). Reaction of **4** with 20 equivalents of α,α′-dichloro-*p*-xylene similarly gave the key benzyl chloride functionalized rhodamine derivative **12** in 88% yield. An excess of the electrophile was employed to prevent *bis*-esterification by α,α′-dichloro-*p*-xylene. Compound **12** could then be cleanly converted to the ester linked compounds **13**–**18** in high yield through reaction with the appropriate carboxylic acid using cesium carbonate and sodium iodide in DMF.[14] This method was applied to the attachment of the *C*-termini of *N*-Boc, *N*-Fmoc and *N*-Cbz protected α-amino acids and a dipeptide to give **13**–**18**. During the course of this study we observed that these compounds were insoluble in diethyl ether which allowed the development of a simple purification procedure, by washing the crude solid residues with diethyl ether and/or

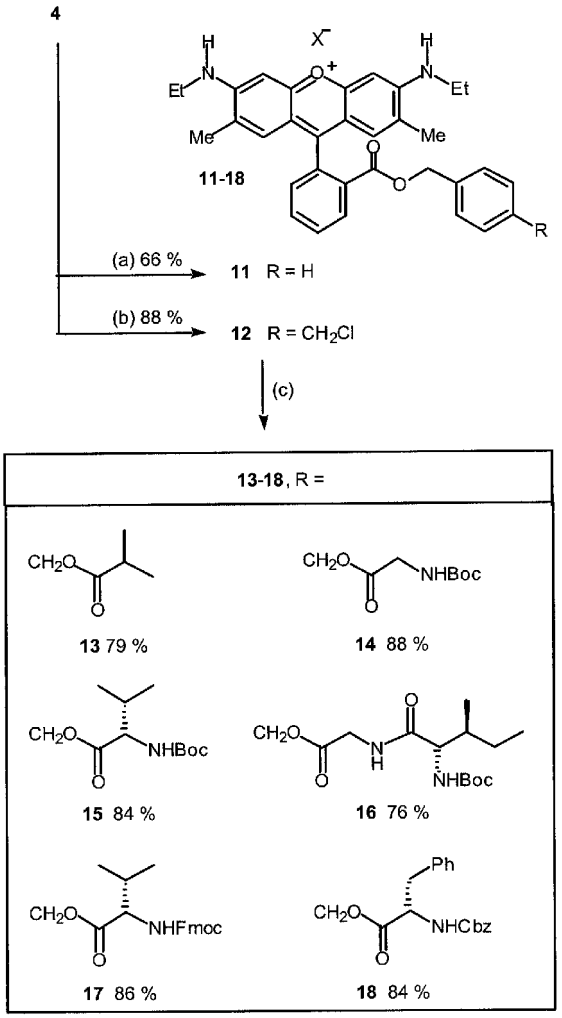


Scheme 1 *Conditions:* (a) reported method:[6] 265–275 °C, 5 h, vacuum; (b) AcCl (1.1 equiv), DMAP (cat.), Et_3N (1.1 equiv), CH_2Cl_2, reflux, overnight; (c) NaH (3.9 equiv), THF, BnOCOCl (3.9 equiv), reflux, 3 h; (d) *i*-Pr_2NEt (1.2 equiv), $BrCH_2CO_2Et$ (1.0 equiv), NaI (cat.), MeCN, reflux, 24 h; (e) *i*-Pr_2NEt (1.2 equiv), $BrCH_2CO_2CH_2Ph$ (1.1 equiv), NaI (cat.), MeCN, reflux, 2.5 h; (f) H_2, 10% Pd/C, EtOH, r.t., 7 h; (g) DCC (1.1 equiv), *N*-hydroxysuccinimide (1.1 equiv), *i*-Pr_2NEt (1.1 equiv), $RNH_2 \cdot HCl$ (1.1 equiv), DMF, r.t., overnight

crystallization using ethanol (or ethyl acetate)–diethyl ether. In addition to the reaction of **12** with carboxylates, we anticipate that the benzyl chloride residue in **12** may also serve as a useful molecular probe for reaction with thiols and other highly nucleophilic functional groups.

Determination of comparative fluorescent properties of some of the compounds prepared above, in relation to rhodamine 6G, were also undertaken. Derivative **6** is not fluorescent by comparison with the strong fluorescence of rhodamine 6G or the derivatives **4**, **5**, **10**, **13** and **15**.[15] X-ray crystallographic analysis of **14** (Figure 2) revealed a clear π-stacked orientation of the xanthenylium ring system with the aromatic ring of the linker.[16,17]

In conclusion, we have demonstrated the use of rhodamine 6G as an inexpensive precursor for the creation of model fluorescent molecular probes. Attachment of the



Scheme 2 *Conditions:* (a) $PhCH_2Br$ (1.0 equiv), NaI (cat.), *i*-Pr_2NEt (1.2 equiv), MeCN, reflux, 4 h; (b) α,α′-dichloro-*p*-xylene (20 mol equiv), NaI (20 mol%), *i*-Pr_2NEt (1.2 equiv), MeCN, reflux, 2.5 h; (c) Cs_2CO_3 (0.65 mol equiv), RCO_2H (1.06 equiv), NaI (1.1 equiv), DMF, r.t., 24 h

rhodamine nucleus to either the *N*- or *C*-termini of amino acids or peptides can be accomplished. The syntheses are short and high yielding and the method developed should be applicable to the formation of a variety of probe molecules and fluorescent reporters.

All reagents were of commercial quality. Anhydrous CH_3CN (CaH_2), DMF (BaO), Et_3N (NaOH), CH_2Cl_2 (P_2O_5) were distilled. Anhydrous benzene, THF and Et_2O were prepared by distillation from Na/benzophenone ketyl under argon. All other solvents and reagents were used as obtained, except hexanes, which were distilled prior to use. All reactions under anhydrous conditions were performed under an atmosphere of nitrogen. Flash column chromatography was performed using silica gel (60 Å, 230-400 mesh, obtained from Whatman Company). Analytical thin-layer chromatography (TLC), was performed on pre-coated silica gel plates, (Alugram SIL G/UV_{254} obtained from Rose Scientific Limited). Solvent systems used to determine R_f values and for chromatography are reported as v/v ratios. Melting points (uncorrected) were determined on a Electrothermal Mod. IA 6304 capillary melting point apparatus. Microanalyses were carried out at Canadian Microanalytical Service Ltd. Low resolution mass spectra (MS) were recorded on a Bell and Howell 21-490 spectrometer. High resolution mass spectra (HRMS) were recorded on a AEI MS3074 spectrometer. Infrared spectra (IR) were recorded on a Perkin-Elmer FT-IR spectrophotometer, with samples loaded as neat films on NaCl plates. ^1H and ^{13}C NMR were recorded on a Varian Unity 200 and 400 spectrometers as solutions in $CDCl_3$ or CD_3OD. Chemical shifts are expressed in δ (ppm) values. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. Fluorescence spectra (excitation at 495 nm) were recorded on a Spex Fluorolog Mod. F 111 spectrophotometer in CH_2Cl_2 Spectranal® (Fluka). The comparative quantum yields relative to Rhodamine 6G were determined according to standard procedures.[18]

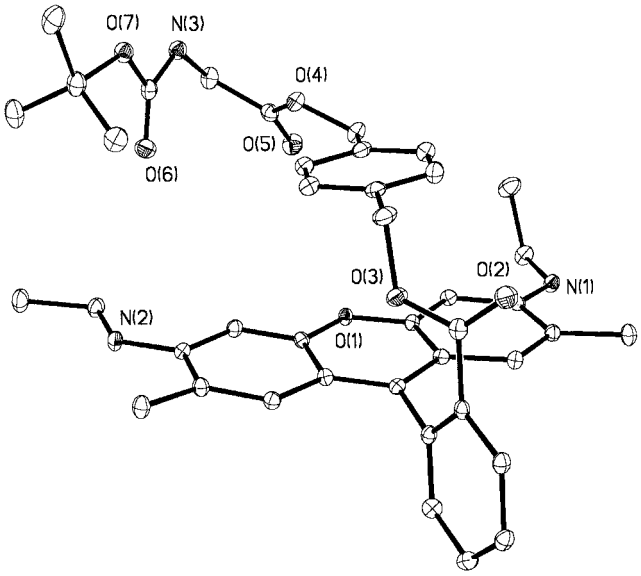


Figure 2 Thermal ellipsoid plot of **14**. Ellipsoids are drawn at the 30% level. Hydrogen atoms and the iodide ion have been omitted for clarity.

Pyrolysis of Rhodamine 6G[6]

Rhodamine 6G (7.0 g, 12.5 mmol, Sigma-Aldrich) was taken in a 250 mL round bottom flask and fitted to a rotary evaporator in the fume hood connected to high vacuum. The flask was immersed in a large oil bath and heated with rotation to 265–275 °C (caution: decomposition was observed if heated to more than 285 °C). After 4–5 h the flask was cooled to give **4** (5.0 g, 86%) as a red powdered solid.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.32 (t, 6 H, *J* = 7.0 Hz, 2 × CH_3), 1.93 (s, 6 H, 2 × $ArCH_3$), 3.23 (m, 4 H, 2 × NCH_2), 3.60 (br, 2 H, 2 × NH), 6.35 and 6.40 (2 × s, 2 × 2 H, ArH), 7.16 (d, 1 H, *J* = 7.7 Hz, ArH), 7.61 (m, 2 H, ArH), 8.02 (d, 1 H, *J* = 6.2 Hz, ArH).

N-Acetyl-rhodamine 5

Acetyl chloride (1.25 g, 15.9 mmol) was added drop-wise to a stirred suspension of **4** (6.0 g, 14.5 mmol), Et_3N (1.61 g, 15.9 mmol) and DMAP (0.178 g, 0.15 mmol) in anhyd CH_2Cl_2 (150 mL) at r.t. under nitrogen. The reaction mixture was refluxed overnight and, after allowing to cool to r.t., Et_2O (200 mL) was added and the reaction mixture filtered. The filtrate was concentrated in vacuo, and the residue purified by flash chromatography (Et_2O–hexane, 4:1) to afford **5** (1.36 g, 21%) as pale yellow needles, mp 268–270 °C.

IR (nujol): 3410, 2923, 2853, 1760, 1651, 1634, 1617, 1524, 1497, 1463, 1376, 1274, 1220, 1106, 1088, 1014, 876, 749 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ (hindered rotation) = 1.13 and 1.31 (2 × t, 6 H, *J* = 7.0 Hz, 2 × CH_3), 1.76 and 1.78 (2 × s, 3 H, CH_3CO), 1.93 and 2.02 (2 × s, 6 H, 2 × $ArCH_3$), 3.13–3.27 (m, 3 H, NCH and NCH_2), 3.72 (br s, 1 H, NH), 4.09 (m, 1 H, NCH), 6.36 and 6.39 (2 × s, 2 H, ArH), 6.65 (d, 1 H, *J* = 2.5 Hz, ArH), 7.00 (s, 1 H, ArH), 7.20 (m, 1 H, ArH), 7.62–7.68 (m, 2 H, ArH), 8.06 (d, 1 H, *J* = 8.0 Hz, ArH).

^{13}C NMR (100 MHz, C_6D_6): δ (hindered rotation) = 13.35, 13.39, 14.48, 16.60, 16.67, 22.38, 22.44, 38.37, 43.06, 43.20, 83.00, 83.11, 96.79, 106.49, 117.92, 118.04, 119.39, 119.42, 120.30, 124.14, 125.24, 127.32, 127.55, 128.94, 128.98, 129.81, 130.67, 130.75, 131.68, 131.72, 134.96, 144.12, 148.94, 148.96, 150.63, 150.66, 151.68, 151.76, 154.59, 154.78, 168.44, 168.85, 169.44, 169.54.

MS (EI): *m*/*z* (%) = 456 (M^+, 13), 441 (2), 412 (70), 397 (98), 383 (78), 369 (13), 353 (21), 342 (53), 326 (100), 299 (19), 282 (10), 170 (10), 70 (71).

HRMS: *m*/*z* calcd for $C_{28}H_{28}N_2O_4$: 456.2049; found: 456.2066.

N-Acetyl-*N*′-benzyloxycarbonyl-rhodamine 6

Benzyl chloroformate (0.29 g, 1.75 mmol) was added drop-wise to a stirred suspension of **5** (0.20 g, 0.44 mmol) and NaH (80% mineral oil, 0.053 g, 1.75 mmol) in anhyd THF (4 mL) at r.t. under nitrogen. The reaction mixture was refluxed for 3 h and then cooled to r.t. H_2O (15 mL) was carefully added, followed by extraction with Et_2O (2 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, concentrated in vacuo and chromatographed on silica gel column (Et_2O) to afford **6** (0.22 g, 85%) as colorless needles, mp 125–127 °C.

IR (nujol): 2923, 2853, 1769, 1704, 1660, 1613, 1490, 1462, 1376, 1273, 1216, 1149, 1084, 748 cm^{-1}.

^1H NMR (200 MHz, $CDCl_3$): δ = 1.13 (m, 6 H, 2 × CH_3), 1.15 and 1.16 (2 × s, 3 H, CH_3CO), 1.75 and 2.04 (s and m, 6 H, 2 × $ArCH_3$), 3.17–4.12 (4 × m, 4 H, 4 × NCH), 5.05–5.16 (m, 2 H, OCH_2Ph), 6.66 and 6.72 (2 × s, 2 H, ArH), 7.04–7.50 (m, 8 H, ArH), 7.68 (br s, 2 H, ArH), 8.08 (d, 1 H, *J* = 6.5 Hz, ArH).

^{13}C NMR (50 MHz, $CDCl_3$): δ (hindered rotation) = 13.36, 13.42, 14.25, 17.42, 17.56, 22.88, 43.36, 45.48, 67.69, 117.34, 118.01, 118.33, 119.47, 124.36, 125.85, 128.40, 128.86, 129.93, 130.61, 131.96, 132.85, 135.84, 137.09, 143.08, 144.02, 149.85, 150.17, 150.27, 153.24, 155.26, 169.55, 169.66, 170.00, 170.22.

MS (EI): *m*/*z* (%) = 590 (M^+, 21), 546 (73), 531 (17), 517 (65), 455 (98), 386 (63), 342 (18), 91 (100).

HRMS: *m*/*z* calcd for $C_{36}H_{34}N_2O_6$: 590.2417; found: 590.2405.

Rhodamine 19 Ethoxycarbonylmethyl Ester Bromide 7[9]

To a stirred mixture of **4** (153 mg, 0.37 mmol), NaI (catalytic amount) in anhyd CH_3CN (3.7 mL) under nitrogen at r.t. was added successively ethyl diisopropylamine (77 μL, 0.44 mmol) and ethyl 2-bromoacetate (41 μL, 0.37 mmol) and then refluxed for 24 h. The reaction mixture was cooled to r.t., concentrated in vacuo and chromatographed on a silica gel column (EtOAc–EtOH, 8:2 to 7:3), to afford a red amorphous solid (191 mg) which was crystallized from EtOH–hexane to give **7** (173 mg, 80%) as red plates, mp 253–256 °C (decomposition), (ethanol–hexane).

IR (film): 3224, 2978, 1729, 1649, 1562, 1528, 1502, 1448, 1367, 1306, 1244, 1211, 1186, 1133, 1092, 1022, 884, 816, 780, 733 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.15 (t, 3 H, *J* = 7.5 Hz, OCH_2CH_3), 1.40 (t, 6 H, *J* = 7.5 Hz, NCH_2CH_3), 2.29 (s, 6 H, $ArCH_3$), 3.55 (qui, 4 H, *J* = 7.5 Hz, NCH_2CH_3), 4.09 (q, 2 H, *J* = 7.5 Hz, OCH_2CH_3), 4.59 (s, 2 H, OCH_2CO_2Et), 6.28 (s, 2 H, ArH), 6.74 (s, 2 H, ArH), 7.03 (t, 2 H, *J* = 5.5 Hz, NH), 7.30 (dd, 1 H, *J* = 1.0, 7.5 Hz, ArH), 7.77 (dt, 1 H, *J* = 1.0, 7.5 Hz, ArH), 7.84 (dt, 1 H, *J* = 1.0, 7.5 Hz, ArH), 8.42 (dd, 1 H, *J* = 1.0, 7.5 Hz, ArH).

^{13}C NMR (50 MHz, $CDCl_3$): δ = 13.73, 18.70, 38.20, 61.30, 93.52, 113.20, 126.12, 128.23, 128.86, 130.00, 130.19, 131.41, 133.18, 134.42, 155.82, 156.05, 157.02, 164.13, 166.65.

MS (EI): *m*/*z* (%) = 500 (M^+, 75), 485 (100), 458 (6), 415 (15), 399 (17), 370 (35), 355 (99), 341 (5), 326 (13), 312 (11).

HRMS: *m*/*z* calcd for $C_{30}H_{32}N_2O_5$: 500.2311; found: 500.2315.

Preparation of 7 from Rhodamine 6G

To a stirred solution of rhodamine 6G **2** (502 mg, 1.05 mmol, from Aldrich) and $LiOH{\cdot}H_2O$ (88 mg, 2.1 mmol) in THF (4 mL) and H_2O (4 mL) was refluxed for 4 h under nitrogen. The solvent was evaporated to dryness in vacuo and acetonitrile (11 mL), NaI (catalytic amount), ethyl diisopropylamine (430 μL, 2.5 mmol) and ethyl 2-bromoacetate (270 μL, 2.4 mmol) were added successively. The reaction mixture was refluxed for 20 h, then cooled to r.t., concentrated in vacuo and purified as described earlier to give **7** (221 mg, 36%).

Rhodamine 19 Benzyloxycarbonylmethyl Ester Bromide 8

Preparation of benzyl 2-bromoacetate: To a stirred solution of benzyl alcohol (2.01 g, 18.6 mmol) and 2,6-lutidine (2.4 mL, 20.4 mmol) in anhyd CH_2Cl_2 (80 mL) at 0 °C under nitrogen was added drop-wise (5 min) bromoacetyl bromide (1.6 mL, 18.6 mmol) and the mixture stirred for 2 h. The solvent was evaporated from the reaction mixture, Et_2O (100 mL) added, filtered and the filtrate was concentrated in vacuo and chromatographed on silica gel column (CH_2Cl_2) to afford **benzyl 2-bromoacetate** (3.87 g, 91%), as an colorless liquid. The material had identical spectral data to those reported,[19] and should be stored in the fridge and used within one week.

To a stirred mixture of **4** (351 mg, 0.85 mmol), NaI (catalytic amount) in anhyd CH_3CN (8.5 mL) under nitrogen at r.t. was added successively ethyl diisopropylamine (177 μL, 1.0 mmol) and benzyl 2-bromoacetate (204 mg, 0.89 mmol) and then refluxed for 2.5 h. The reaction mixture was cooled to r.t., concentrated in vacuo and chromatographed on silica gel column (EtOAc–EtOH, 8:2 to 7:3) to afford **8** (418 mg, 77%) as gold needles, mp 220–224 °C (EtOAc–EtOH).

IR (film): 3226, 3024, 2976, 1731, 1650, 1606, 1564, 1527, 1503, 1447, 1366, 1305, 1186, 1132, 1091, 1022, 816, 733, 698, 667 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.39 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.26 (d, 6 H, *J* = 1.0 Hz, $ArCH_3$), 3.53 (m, 4 H, NCH_2), 4.63 (s, 2 H, OCH_2COCH_2Ph), 5.05 (s, 2 H, OCH_2Ph), 6.62 (s, 2 H, ArH), 6.73 (d, 2 H, *J* = 1.0 Hz, ArH), 6.94 (br s, 2 H, NH), 7.19–7.22 (m, 2 H, ArH), 7.26–7.29 (m, 4 H, ArH), 7.75 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.82 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.39 (dd, 1 H, *J* = 1.1, 7.5 Hz, ArH).

^{13}C NMR (50 MHz, CD_3OD): δ = 14.08, 17.60, 39.43, 62.55, 67.71, 94.87, 114.86, 126.83, 128.72, 129.20, 129.44, 130.04, 130.59, 131.47, 131.77, 132.49, 134.56, 135.70, 136.86, 157.62, 158.83, 158.92, 166.04, 168.59.

MS (EI): *m*/*z* (%) = 562 (M^+, 31), 547 (52), 415 (14), 370 (32), 369 (31), 355 (100), 341 (5), 339 (15), 326 (15), 311 (12), 297 (6), 149 (18), 107 (27), 91 (87).

HRMS: *m*/*z* calcd for $C_{35}H_{34}N_2O_5$: 562.2468; found: 562.2466.

Anal. Calcd for $C_{35}H_{35}BrN_2O_5$: C, 65.32; H, 5.48; N, 4.35. Found: C, 64.98; H, 5.42; N, 4.29.

Rhodamine 19 Hydroxycarbonylmethyl Ester Bromide 9

A mixture of **8** (197 mg, 0.31 mmol) and 10% palladium on carbon (58 mg) in absolute EtOH (8 mL) was vigorously stirred under hy-

drogen atmosphere (balloon) for 24 h at r.t. The mixture was filtered, concentrated in vacuo and crystallized from EtOH–EtOAc to afford **9** (134 mg, 79%) as green plates, mp 106–360 °C (slow decomposition), (EtOH–EtOAc).

IR (film): 3270, 2970, 1725, 1647, 1606, 1529, 1501, 1446, 1305, 1266, 1242, 1185, 1134, 1089, 1021 cm^{-1}.

^1H NMR (400 MHz, CD_3OD): δ = 1.36 (t, 6 H, *J* = 7.5 Hz, NCH_2CH_3), 2.12 (d, 6 H, *J* = 1.0 Hz, $ArCH_3$), 3.52 (q, 4 H, *J* = 7.5 Hz, NCH_2CH_3), 4.88 (s, 2 H, OCH_2CO_2H), 6.87 (d, 2 H, *J* = 1.0 Hz, ArH), 6.91 (s, 2 H, ArH), 7.42 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.83 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.89 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.41 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH).

^{13}C NMR (50 MHz, CD_3OD): δ = 14.09, 17.66, 39.43, 62.37, 94.85, 114.83, 126.79, 130.02, 130.79, 131.44, 131.59, 131.72, 131.84, 132.49, 134.46, 135.60, 157.55, 158.86, 166.05, 170.26.

MS (EI): *m*/*z* (%) = 472 (M^+, 55), 457 (80), 444 (15), 429 (20), 415 (60), 413 (100), 399 (45).

HRMS: *m*/*z* calcd for $C_{28}H_{28}N_2O_5$: 472.1998; found: 472.1983.

Rhodamine 19 (L-Methyl-phenylalaninyl)-carbonylmethyl Ester Bromide 10[10]

To a stirred solution of the crude solid residue of **9** [prepared as reported above starting from **8** (62.0 mg, 0.10 mmol)] in anhyd DMF (2.5 mL) at r.t. under nitrogen, was successively added *N*-hydroxysuccinimide (12.6 mg, 0.11 mmol) and a solution of 1,3-dicyclohexylcarbodiimide (22.6 mg, 0.11 mmol) in anhyd CH_2Cl_2 (1 mL). After 30 min ethyl diisopropylamine (19 μL, 0.11 mmol) and L-phenylalanine methyl ester hydrochloride (23.7 mg, 0.11 mmol) were successively added and the mixture was stirred overnight. The reaction mixture was diluted with CH_2Cl_2 (80 mL), washed with H_2O (2 × 50 mL), dried ($MgSO_4$), concentrated in vacuo and chromatographed on silica gel column (CH_2Cl_2–MeOH, 9:1) to afford **10** (47.3 mg, 69%) as red solid, mp 145–169 °C (decomposition), (EtOH–EtOAc–Et_2O; red plates).

IR (film): 3241, 3024, 2976, 2931, 2853, 1732, 1650, 1607, 1529, 1501, 1448, 1367, 1306, 1186, 1140, 1090, 1022, 814, 733, 701, 667 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.35 (q, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.22 (s, 3 H, $ArCH_3$), 2.24 (s, 3 H, $ArCH_3$), 3.04 (dd, 1 H, *J* = 6.0, 13.5 Hz, $PhCH_2CHN$), 3.09 (dd, 1 H, *J* = 6.0, 13.5 Hz, $PhCH_2CHN$), 3.44–3.52 (br, 4 H, NCH_2CH_3), 3.69 (s, 3 H, CO_2CH_3), 4.50 (d, 1 H, *J* = 15.5 Hz, OCH_2CO), 4.54 (d, 1 H, *J* = 15.5 Hz, OCH_2CO), 4.78 (dd, 1 H, *J* = 6.0, 13.5 Hz, $PhCH_2CHN$), 6.59 (s, 1 H, ArH), 6.62 (s, 1 H, ArH), 6.66 (s, 1 H, ArH), 6.69 (s, 1 H, ArH), 6.68–6.71 (m, 1 H, ArH), 6.92 (br s, 1 H, NH), 7.00–7.03 (m, 3 H, ArH, NH), 7.20–7.21 (m, 3 H, ArH), 7.29 (d, 1 H, *J* = 7.0 Hz, ArH), 7.73 (t, 1 H, *J* = 7.5 Hz, ArH), 7.84 (t, 1 H, *J* = 7.5 Hz, ArH), 8.20 (d, 1 H, *J* = 7.5 Hz, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.89, 15.25, 18.41, 18.53, 24.94, 25.58, 33.87, 37.45, 38.47, 49.00, 52.55, 52.84, 63.42, 65.84, 93.89, 113.39, 113.41, 126.06, 126.11, 127.16, 128.29, 128.46, 128.56, 129.23, 130.28, 130.39, 131.32, 133.58, 134.87, 135.49, 155.83, 155.89, 156.28, 157.04, 157.09, 163.46, 165.90, 171.47.

MS (EI): *m*/*z* (%) = 633 (M^+, 20), 618 (37), 574 (6), 413 (70), 399 (21), 369 (24), 355 (72), 339 (15), 326 (17), 224 (16), 187 (17).

HRMS: *m*/*z* calcd for $C_{38}H_{39}N_3O_6$: 633.2839; found: 633.2809.

Rhodamine 19 Benzyl Ester Bromide 11

To a stirred mixture of **4** (157 mg, 0.38 mmol), NaI (catalytic amount) in anhyd CH_3CN (3.8 mL) under nitrogen at r.t. was added successively ethyl diisopropylamine (79 μL, 0.45 mmol) and benzyl bromide (45 μL, 0.38 mmol) and then refluxed for 4 h. The reaction mixture was cooled to r.t., concentrated in vacuo and chromatographed on silica gel column (EtOAc–EtOH, 9:1 to 8:2) to afford **11** (145 mg, 66%) as red solid; mp 264-285 °C (decomposition) (EtOH–hexane, red plates).

IR (film): 3233, 3024, 2976, 1718, 1649, 1607, 1561, 1528, 1500, 1449, 1367, 1306, 1187, 1130, 1088, 1022, 815, 733, 698 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.46 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.30 (s, 6 H, $ArCH_3$), 3.62 (quintet, 4 H, *J* = 7.0 Hz, NCH_2CH_3), 4.96 (s, 2 H, OCH_2Ph), 6.57 (s, 2 H, ArH), 6.72 (d, 2 H, *J* = 1.0 Hz, ArH), 6.93 (dd, 2 H, *J* = 1.5, 7.5 Hz, ArH), 7.15 (t, 2 H, *J* = 5.5 Hz, NH), 7.21 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.22 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.26–7.32 (m, 2 H, ArH), 7.76 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.81 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.36 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH).

^{13}C NMR (50 MHz; $CDCl_3$) δ = 13.88, 18.77, 38.34, 67.37, 93.63, 113.26, 126.15, 128.12, 128.26, 128.38, 129.99, 131.38, 132.76, 133.79, 134.21, 155.81, 156.28, 156.88, 164.91.

MS (EI): *m*/*z* (%) = 504 (M^+, 35), 489 (38), 413 (13), 370 (42), 369 (42), 355 (100), 339 (12), 326 (15), 311 (12).

HRMS: *m*/*z* calcd for $C_{33}H_{32}N_2O_3$: 504.2413; found: 504.2431.

Rhodamine 19 4-Chloromethyl-1-phenylmethyl Ester Chloride 12

To a stirred mixture of **4** (1.12 g, 2.70 mmol), NaI (81 mg, 0.54 mmol) in anhyd CH_3CN (20 mL) under nitrogen at r.t. was added successively ethyl diisopropylamine (565 μL, 3.24 mmol) and α,α′-dichloro-*p*-xylene (9.47 g, 54.1 mmol) and then refluxed for 2.5 h. The reaction mixture was warmed to r.t., concentrated in vacuo and the solid residue washed successively with Et_2O (5 × 30 mL), EtOAc (2 × 20 mL) and Et_2O (1 × 20 mL), and crystallized from EtOH–EtOAc–Et_2O to give **12** (1.40 g, 88%) as a red powdered solid, mp > 345 °C (decomposition).

IR (film): 3222, 3024, 2976, 1717, 1649, 1606, 1566, 1528, 1501, 1448, 1367, 1306, 1186, 1130, 1087, 1022, 821, 733 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.42 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.25 (s, 6 H, $ArCH_3$), 3.56 (quintet, 4 H, *J* = 7.0 Hz, NCH_2CH_3), 4.57 (s, 2 H, $ArCH_2Cl$), 4.91 (s, 2 H, OCH_2Ar), 6.54 (s, 2 H, ArH), 6.67 (s, 2 H, ArH), 6. 86 (d, 2 H, *J* = 8.0 Hz, ArH), 7.19 (d, 2 H, *J* = 8.0 Hz, ArH), 7.23–7.24 (m, 1 H, ArH), 7.32 (br t, 2 H, *J* = 7.0 Hz, NH), 7.72 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.77 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.32 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH).

^{13}C NMR (100 MHz, CD_3OD): δ = 14.15, 17.66, 39.46, 46.59, 67.97, 94.91, 114.63, 126.81, 129.66, 129.75, 129.80, 131.44, 131.51, 132.42, 134.12, 134.80, 135.97, 139.33, 157.43, 158.57, 158.66, 166.58.

MS (EI): *m*/*z* (%) = 552 (M^+, 58), 537 (70), 516 (34), 501 (28), 413 (25), 399 (17), 369 (30), 355 (70), 339 (28), 326 (23), 312 (19), 174 (26), 139 (100).

HRMS: *m*/*z* calcd for $C_{34}H_{33}ClN_2O_3$: 552.2179; found: 552.2183.

Attachment of Carboxylic Acid Derivatives to Rhodamine Derivative 12; General Procedure[14]

Rhodamine 19 4-(2′-Methyl-propionyl)-oxymethyl-1-phenylmethyl Ester Iodide 13

A suspension of isobutyric acid (162 mg, 0.18 mmol) and Cs_2CO_3 (36.1 mg, 0.11 mmol) in anhyd DMF (2 mL) was stirred at r.t. for 1 h, and then evaporated at 40–45 °C under reduced pressure. The residue was dissolved in anhyd DMF (2 mL) and NaI (27.6 mg, 0.18 mmol) and **12** (102.0 mg, 0.17 mmol) were successively added. After 24 h the mixture was partitioned between CH_2Cl_2 (70 mL) and H_2O (50 mL). The organic layer was washed with H_2O (4 × 50 mL), dried ($MgSO_4$), concentrated in vacuo and crystallized from EtOH–Et_2O (1:10, 22 mL) to give **13** (100.1 mg, 79%) as red plates, mp 136–138 °C.

IR (film): 3244, 3024, 2974, 2935, 2874, 1721, 1649, 1605, 1559, 1527, 1499, 1448, 1367, 1306, 1186, 1129, 1077, 1021, 885, 815, 732 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.16 [d, 6 H, *J* = 7.0 Hz, $CH(CH_3)_2$], 1.37 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.22 (s, 6 H, $ArCH_3$), 2.58 (heptet, 1 H, *J* = 7.0 Hz, $CHMe_2$), 3.56 (quintet, 4 H, *J* = 7.0 Hz, NCH_2CH_3), 4.88 (s, 2 H, $ArCO_2CH_2Ar'$), 5.06 (s, 2 H, $ArCH_2OCOCHMe_2$), 6.53 (s, 2 H, ArH), 6.65 (s, 2 H, ArH), 6.82 (br t, 2 H, *J* = 5.5 Hz, NH), 6.87 (d, 2 H, *J* = 7.5 Hz, ArH), 7.13 (d, 2 H, *J* = 7.5 Hz, ArH), 7.19 (d, 1 H, *J* = 7.5 Hz, ArH), 7.69 (t, 1 H, *J* = 7.5 Hz, ArH), 7.75 (t, 1 H, *J* = 7.5 Hz, ArH), 8.27 (d, 1 H, *J* = 7.5 Hz, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.87, 18.84, 19.01, 33.84, 38.21, 65.31, 66.87, 93.63, 113.18, 125.90, 127.83, 128.24, 128.28, 129.50, 129.93, 130.03, 131.32, 132.91, 133.67, 133.99, 136.46, 155.54, 156.69, 156.80, 164.72, 176.72.

MS (EI): *m*/*z* (%) = 604 (M^+, 18), 589 (32), 533 (42), 413 (11), 370 (41), 369 (41), 355 (100), 326 (15), 311 (12), 191 (52), 104 (30).

HRMS: *m*/*z* calcd for $C_{38}H_{40}N_2O_5$: 604.2937; found: 604.2956.

Rhodamine 19 4-(*N*-*t*-Butoxycarbonyl-glycinyl-oxymethyl)-1-phenylmethyl Ester Iodide 14

Prepared as described for **13** using *N*-*t*-Boc-Glycine (38.5 mg, 0.22 mmol). Compound **14** (148.5 mg, 88%) was obtained as red plates, mp >113 °C (decomposition, gas liberation, EtOH–Et_2O).

IR (film): 3252, 3024, 2976, 2928, 1715, 1651, 1607, 1558, 1529, 1504, 1448, 1367, 1307, 1186, 1089, 1022, 815, 733 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 1.37 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 1.37 [s, 9 H, $C(CH_3)_3$], 2.20 (s, 6 H, $ArCH_3$), 3.56 (quintet, 4 H, *J* = 7.0 Hz, NCH_2Me), 3.95 (d, 2 H, *J* = 5.5 Hz, NCH_2CO), 4.89 (s, 2 H, $ArCO_2CH_2Ar'$), 5.11 (s, 2 H, $ArCH_2OCOCH_2NHBoc$), 5.20 (t, 1 H, *J* = 5.5 Hz, NHBoc), 6.56 (s, 2 H, ArH), 6.64 (s, 2 H, ArH), 6.82 (br t, 2 H, *J* = 5.0 Hz, NH), 6.87 (d, 2 H, *J* = 7.5 Hz, ArH), 7.12 (d, 2 H, *J* = 7.5 Hz, ArH), 7.19 (d, 1 H, *J* = 7.5 Hz, ArH), 7.69 (dt, 1 H, *J* = 1.0, 6.5 Hz, ArH), 7.75 (dt, 1 H, *J J* = 1.0, 6.5 Hz, ArH), 8.27 (d, 1 H, *J* = 7.5 Hz, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.90, 18.93, 28.15, 38.26, 42.37, 66.22, 66.76, 79.85, 93.68, 113.20, 125.92, 128.08, 128.28, 128.35, 129.58, 129.96, 130.05, 131.33, 132.92, 133.66, 134.43, 135.52, 155.59, 155.68, 156.71, 156.85, 164.76, 170.14.

MS (EI): *m*/*z* (%) = 691 (M^+, 15), 676 (7), 645 (7), 636 (18), 617 (7), 602 (12), 590 (8), 533 (17), 517 (98), 501 (100), 473 (13), 442 (23).

HRMS: *m*/*z* calcd for $C_{41}H_{45}N_3O_7$: 691.3258; found: 691.3239.

Anal. Calcd for $C_{41}H_{46}IN_3O_7$: C, 60.07; H, 5.66; N, 5.13. Found: C, 60.32; H, 5.79; N, 4.90.

Rhodamine 19 4-(*N*-*t*-Butoxycarbonyl-l-valinyl-oxymethyl)-1-phenylmethyl Ester Iodide 15

Prepared as described for **13** using *N*-*t*-Boc-L-valine (47.8 mg, 0.22 mmol). Compound **15** (148.8 mg, 84%) was obtained as red plates, mp 158–161 °C (EtOH–Et_2O).

IR (film): 3241, 2963, 2932, 2874, 1723, 1651, 1607, 1557, 1526, 1504, 1449, 1367, 1306, 1186, 1129, 1075, 1022, 815, 739 cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$): δ = 0.87 [d, 3 H, *J* = 7.0 Hz, $CH(CH_3)_2$], 0.95 [d, 3 H, *J* = 7.0 Hz, $CH(CH_3)_2$], 1.40 [s, 9 H, $C(CH_3)_3$], 1.415 (t, 3 H, *J* = 7.0 Hz, NCH_2CH_3), 1.422 (t, 3 H, *J* = 7.0 Hz, NCH_2CH_3), 2.15 (m, 1 H, $CHMe_2$), 2.21 (s, 3 H, $ArCH_3$), 2.22 (s, 3 H, $ArCH_3$), 3.59 (quintet, 4 H, *J* = 7.0 Hz, NCH_2CH_3), 4.23 (dd, 1 H, *J* = 5.0, 9.0 Hz, CHNHBoc), 4.91 (d, 1 H, *J* = 12.0 Hz, $ArCO_2CH_2Ar'$), 4.97 (d, 1 H, *J* = 12.0 Hz, $ArCO_2CH_2Ar'$), 5.01 (d, 1 H, *J* = 9.0 Hz, NHBoc), 5.08 (d, 1 H, *J* = 12.5 Hz, $ArCH_2OCOCHNHBoc$), 5.18 (d, 1 H, *J* = 12.5 Hz, $ArCH_2OCOCHNHBoc$), 6.62 (s, 2 H, ArH), 6.68 (br s, 2 H, NH), 6.70 (d, 2 H, *J* = 1.0 Hz, ArH), 6.94 (d, 2 H, J = 8.0 Hz, ArH), 7.17 (d, 2 H, *J* = 8.0 Hz, ArH), 7.23 (d, 1 H, *J* = 8.0 Hz, ArH), 7.72 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.78 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.32 (dd, 1 H, *J* = 1.0, 7.5 Hz, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.75, 14.97, 17.30, 18.82, 18.91, 18.95, 27.97, 30.91, 38.06, 58.35, 65.52, 66.05, 66.63, 79.55, 93.51, 113.02, 125.81, 128.03, 128.11, 128.16, 129.35, 129.85, 129.93, 131.17, 132.84, 133.56, 134.29, 135.40, 155.36, 155.43, 155.45, 156.57, 156.70, 164.59, 171.95.

MS (EI): *m*/*z* (%) = 733 (M^+, 4), 676 (5), 644 (5), 533 (9), 413 (9), 370 (40), 369 (42), 355 (100), 326 (14), 311 (12), 264 (48).

HRMS: *m*/*z* calcd for $C_{44}H_{51}N_3O_7$: 733.3727; found: 733.3714.

Anal. Calcd for $C_{44}H_{52}IN_3O_7$: C, 61.32; H, 6.08; N, 4.88. Found: C, 61.19; H, 6.00; N, 4.83.

Rhodamine 19 4-(*N*-*t*-Butoxycarbonyl-l-isoleucinyl-glycinyl-oxymethyl)-1-phenylmethyl Ester Iodide 16

Prepared as described for **13** using *N*-*t*-Boc-L-isoleucine-glycine (63.3 mg, 0.22 mmol). Compound **16** (146.7 mg, 76%) was obtained as red plates, mp >110 °C (decomposition, gas liberation, EtOH–hexane).

IR (film): 3254, 2973, 2928, 1717, 1650, 1607, 1558, 1528, 1500, 1449, 1366, 1306, 1185, 1129, 1089, 1021, 814, 733 cm^{-1}.

^1H NMR (400 MHz, CD_3OD): δ = 0.87 (t, 3 H, *J* = 7.5 Hz, $CHCH_2CH_3$), 0.94 (d, 3 H, *J* = 6.5 Hz, $CHCH_3$), 1.12–1.57 (m, 2 H, $CHCH_2CH_3$), 1.38 (t, 6 H, *J* = 7.5 Hz, NCH_2CH_3), 1.42 [s, 9 H, $C(CH_3)_3$], 1.81 (m, 1 H, CHMe), 2.07 (s, 6 H, $ArCH_3$), 3.54 (q, 4 H, *J* = 7.5 Hz, NCH_2CH_3), 3.99 (d, 1 H, *J* = 7.0 Hz, CHNHBoc), 4.03 (d, 1 H, *J* = 17.5 Hz, CH_2NH), 4.13 (d, 1 H, *J* = 17.5 Hz, CH_2NH), 4.86 (s, 2 H, $ArCO_2CH_2Ar'$), 5.17 (s, 2 H, $ArCH_2OCOCH_2NH$), 6.77 (s, 6 H, ArH), 7.14 (d, 2 H, *J* = 8.0 Hz, ArH), 7.35 (d, 1 H, *J* = 6.5 Hz, ArH), 7.80 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 7.85 (dt, 1 H, *J* = 1.5, 7.5 Hz, ArH), 8.31 (dd, 1 H, *J* = 1.0, 7.5 Hz, ArH).

^{13}C NMR (100 MHz, CD_3OD): δ = 11.67, 14.24, 15.95, 17.77, 25.67, 28.70, 38.46, 39.49, 42.08, 60.57, 67.22, 68.00, 80.53, 95.04, 114.60, 126.51, 129.09, 129.59, 129.76, 129.89, 131.38, 131.43, 131.49, 132.35, 134.10, 134.76, 135.77, 137.40, 157.38, 157.78, 158.53, 158.56, 166.54, 170.86, 174.96.

MS (EI): *m*/*z* (%) = 516 (2), 413 (7), 370 (45), 369 (46), 355 (100), 340 (9), 326 (18), 311 (16), 230 (30).

MS (Electrospray): *m*/*z* (%) = 806 (MH^+, 20), 539 (8), 467 (5), 215 (30), 150 (55), 136 (100).

Anal. Calcd for $C_{47}H_{57}IN_4O_8$: C, 60.51; H, 6.16; N, 6.01. Found: C, 60.20; H, 6.10; N, 5.54.

Rhodamine 19 4-(*N*-Fluorenylmethoxycarbonyl-l-valinyl-oxymethyl)-1-phenylmethyl Ester Iodide 17

Prepared as described for **13** using *N*-Fmoc-L-valine (76.1 mg, 0.22 mmol). Compound **17** (174.8 mg, 86%) was obtained as red plates by crystallization from EtOAc–Et_2O), mp 129–140 °C (slow decomposition).

IR (film): 3243, 3024, 2972, 1716, 1650, 1606, 1557, 1526, 1504, 1449, 1368, 1305, 1186, 1129, 1089, 1022, 814, 735 cm^{-1}.

^1H NMR (400 MHz, CD_3OD): δ = 0.97 [d, 3 H, *J* = 7.0 Hz, $CH(CH_3)_2$], 0.98 [d, 3 H, *J* = 7.0 Hz, $CH(CH_3)_2$], 1.33 (t, 3 H, *J* = 7.0 Hz, NCH_2CH_3), 1.34 (t, 3 H, *J* = 7.0 Hz, NCH_2CH_3), 1.99 (s, 3 H, $ArCH_3$), 2.00 (s, 3 H, $ArCH_3$), 2.20 (octet, 1 H, *J* = 7.0 Hz, $CHMe_2$), 3.47 (m, 4 H, NCH_2CH_3), 3.98 (t, 1 H, *J* = 7.0 Hz, $CHCH_2OCOCHNH$), 4.17 (dd, 1 H, *J* = 7.5, 10.5 Hz, $CHCH_2OCOCHNH$), 4.20 (d, 1 H, *J* = 6.0 Hz, CHNHFmoc), 4.28 (dd, 1 H, *J* = 7.0, 10.5 Hz, $CHCH_2OCOCHNH$), 4.74 (s, 2 H, $ArCO_2CH_2ArCH_2OCOCHNHFmoc$), 5.08 (d, 1 H, *J* = 12.5 Hz, $ArCH_2OCOCHNHFmoc$), 5.18 (d, 1 H, *J* = 12.5 Hz,

ArCH_2OCOCHNHFmoc), 6.63–6.74 (m, 6 H, ArH), 7.09 (d, 2 H, *J* = 8.0 Hz, ArH), 7.14 (d, 1 H, *J* = 7.5 Hz, ArH), 7.16 (d, 1 H, *J* = 7.5 Hz, ArH), 7.20 (d, 1 H, *J* = 7.0 Hz, ArH), 7.24 (d, 1 H, *J* = 7.5 Hz, ArH), 7.26 (d, 1 H, *J* = 7.5 Hz, ArH), 7.49 (d, 1 H, *J* = 7.5 Hz, ArH), 7.53 (d, 1 H, *J* = 7.5 Hz, ArH), 7.64 (d, 1 H, *J* = 2.0 Hz, ArH), 7.65 (d, 1 H, *J* = 2.0 Hz, ArH), 7.75 (t, 1 H, *J* = 7.5 Hz, ArH), 7.80 (t, 1 H, *J* = 7.5 Hz, ArH), 8.26 (dd, 1 H, *J* = 1.5, 7.5 Hz, ArH).

^{13}C NMR (100 MHz, CD_3OD): δ = 14.28, 14.36, 17.82, 18.71, 19.76, 31.80, 39.43, 39.48, 61.42, 67.20, 67.91, 94.91, 95.08, 114.48, 114.56, 120.80, 120.83, 126.03, 126.13, 126.63, 128.00, 128.04, 128.63, 128.67, 129.32, 129.40, 129.46, 129.59, 129.72, 131.27, 131.34, 131.43,. 132.32, 134.07, 134.66, 135.69, 137.28, 142.23, 142.33, 144.78, 145.23, 157.14, 157.22, 158.27, 158.26, 158.32, 158.63, 166.42, 173.42.

MS (EI): *m*/*z* (%) = 618 (6), 533 (5), 413 (7), 371 (7), 355 (76), 326 (11), 178 (100).

MS (Electrospray): *m*/*z* (%) = 856 (MH^+, 10), 538 (7), 215 (15), 150 (50), 136 (100).

Anal. Calcd for $C_{54}H_{54}IN_3O_7$: C, 65.92; H, 5.53; N, 4.27. Found: C, 65.57; H, 5.84; N, 4.36.

Rhodamine 19 4-(*N*-Benzyloxycarbonyl-l-phenylalaninyl-oxymethyl)-1-phenylmethyl Ester Iodide 18

Prepared as described for **13** using *N*-Cbz-L-phenylalanine (28.7 mg, 0.10 mmol). Compound **18** (71.2 mg, 84%) was obtained as red plates, mp 122–125 °C (EtOH–EtOAc–Et_2O).

IR (film): 3248, 3029, 2975, 1716, 1650, 1606, 1503, 1448, 1367, 1306, 1186, 1130, 1083, 1022, 886, 815, 734, 700 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.38 (t, 6 H, *J* = 7.0 Hz, NCH_2CH_3), 2.19, 2.22 and 2.23 (3 × s, 6 H, $ArCH_3$), 3.10 (m, 2 H, $CHCH_2Ph$), 3.55 (br s, 4 H, NCH_2CH_3), 4.65 (dd, 1 H, *J* = 6.5, 13.5 Hz, C*H*NHCbz), 4.85–5.14 (m, 6 H, $ArCH_2$ and $NHCH_2OBn$), 5.30 (d, 1 H, *J* = 8.0 Hz, $ArCH_2$), 6.57–6.60 (m, 2 H, ArH), 6.66 (d, 2 H, *J* = 13.0 Hz, ArH), 6.79–6.85 (m, 2 H), 6.93 (d, 2 H, *J* = 7.5 Hz), 7.00 (d, 2 H, *J* = 7.0 Hz), 7.09 (d, 2 H, *J* = 7.5 Hz), 7.17–7.26 (m, 9 H), 7.71 (t, 1 H, *J* = 7.5 Hz, ArH), 7.77 (t, 1 H, *J* = 7.5 Hz, ArH), 8.30 (d, 1 H, *J* = 7.5 Hz, ArH).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.93, 15.17, 18.74, 18.85, 18.91, 38.00, 38.36, 45.83, 54.85, 65.75, 66.58, 66.81, 66.87, 93.76, 93.79, 113.31, 125.90, 125.94, 127.06, 127.89, 128.09, 128.33, 128.39, 128.54, 128.63, 128.76, 129.13, 129.61, 130.07, 130.11, 131.36, 133.00, 133.81, 134.68, 135.29, 135.33, 135.94, 155.61, 155.64, 155.67, 156.79, 156.91, 156.93, 164.78, 171.33.

MS (EI): *m*/*z* (%) = 501 (6), 370 (18), 369 (19), 355 (60), 326 (9), 139 (13), 108 (27), 91 (100).

MS (Electrospray): *m*/*z* (%) = 817 (MH^+, 100), 645 (20), 553 (30).

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(15) The *relative* quantum yields at 495 nm of derivatives **10**, **13** and **15** were 1.26, 1.03 and 0.95 × that of rhodamine 6G respectively (see Ref.[18] for the method used).

(16) The crystals of compound **14** were obtained by crystallization from Et_2O–EtOH: $C_{41}H_{46}IN_3O_7$, M = 819.71,

triclinic, space group P–1, a = 12.6230(2), b = 13.4724(3), c = 13.5600(3) Å, α = 101.865(1), β = 107.003(1), γ = 113.602(1)°, V = 1878.77(7) Å^3, Z = 2, D_c = 1.449 gcm^{-3}, μ = 0.907 mm^{-1}, F(000) = 844, crystal dimensions 0.35 × 0.32 × 0.28 mm^3. The intensities of 28224 reflections were measured on a Nonius Kappa-CCD diffractometer (MoKα radiation, T = 100.0(1) K, 4.19 < θ < 30.48, 10822 unique reflections). The structure was solved and refined using the SHELXTL package (see Ref.[17]). Non-hydrogen atoms were assigned anisotropic thermal parameters. Hydrogen atoms were included in calculated positions and treated as riding atoms. The refinement which included 469 parameters, converged with *R*1[I>2σ(I)] = 0.0375 (for 8449 reflections with I>2σ(I)) and *wR*2 (all unique data) = 0.0970. Atomic coordinates and further crystallographic details have been deposited at the Cambridge Crystallographic Data Centre, deposition number CCDC–191818, and copies of this data can be obtained in application to CCDC, 12, Union Road, Cambridge CB2 1EZ, UK. [Fax: +44(1223)336033; E-mail: deposit@ccdc.cam.ac.uk].

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