Efficient Synthesis of Fluorophore-Linked Maleimide Derivatives

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Received 4 December 1997

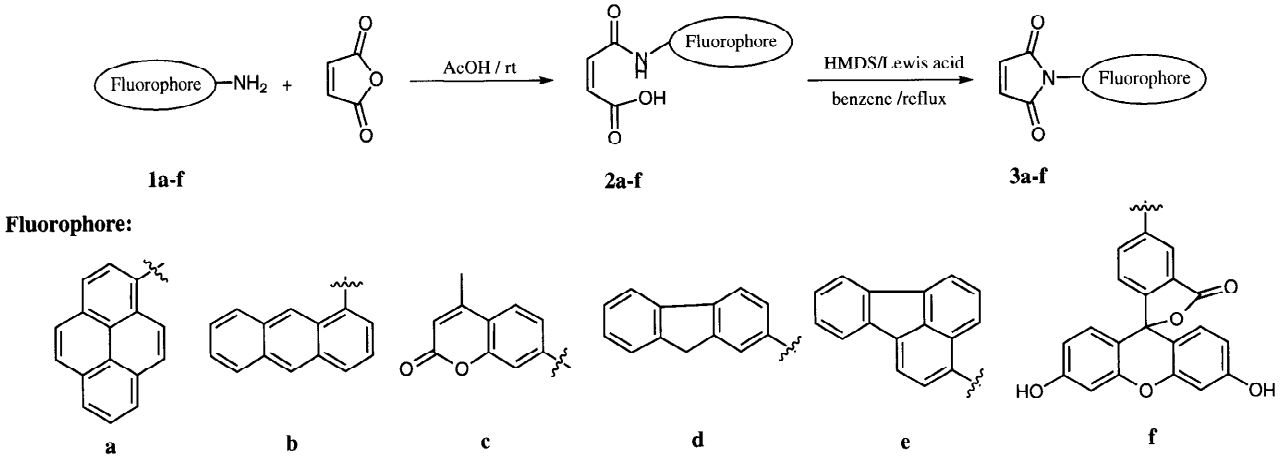
Abstract: Lewis acid and hexamethyldisilazane promoted efficient synthesis of various fluorophore-linked maleimide derivatives from maleic anhydride and amines is described.

Key words: Lewis acid, hexamethyldisilazane, fluorescence, fluorophore, maleamic acid, maleimide

Fluorescence-based analytical techniques are valuable tools in different branches of chemistry and biology.[1] In order to overcome many disadvantages in the use of highly hazardous radioisotopic analytical techniques, extensive research of ours and others in this area led to the synthesis of a number of new fluorescent compounds for efficient detection of biological substrates at low concentrations.[2] In particular, fluorogenic maleimide derivatives are attractive because of their useful properties, such as high reactivity towards a thiol group, fluorescence off-on switching nature, and the long-lived fluorescence.[3] Extensive investigation by Kanaoka and co-workers in the area of fluorescent thiol probes enhanced the scope of fluorophore-linked maleimide derivatives to various biological applications.[4] In spite of their widespread use as potential analytical reagents, there are only a few reports on the synthesis of fluorophore-linked maleimide derivatives.[3] Moreover, the traditional synthetic procedure for *N*-alkyl- and *N*-arylmaleimide derivatives, involving a key amic acid cyclization step with acetic anhydride fused sodium acetate, often gives low yields of fluorophore-linked maleimide derivatives due to the harsh reaction conditions.[3e] For example, our efforts to synthesize *N*-(5-fluoresceinyl)maleimide by this method resulted in extensive formation of undesired products such as *O*-acetylated and acetic acid addition products along with a trace of the desired maleimide derivative. Furthermore, laborious purification of the products from a large excess of reagents and byproducts makes this methodology undesirable. Although some improved methods for the synthesis of maleimide derivatives have appeared recently, these methods are not suitable for the synthesis of fluorophore-linked maleimide derivatives[5a] or they give only moderate yields of the desired products.[5b]

Recently, we reported a Lewis acid and hexamethyldisilazane promoted efficient one-pot method for the synthesis of *N*-alkyl- and *N*-arylimide derivatives possessing various functional groups.[6] In order to enhance the scope and the generality of the methodology, now we report herein the synthesis of various fluorophore-linked maleimide derivatives **3a–f** as shown in the Scheme.

Due to the low solubility of the amines **1a–f** in many organic solvents, our efforts to synthesize maleimide derivatives **3a–f** in a one-pot process were unsuccessful. In particular, formation of fluorophore-linked amic acid in solvents such as acetone, THF, or DMF was very slow. Moreover, cyclization of amic acids **2a–f** in these solvents gave low yields of maleimide derivatives even after heating for extended periods. In order to circumvent these difficulties, we further investigated the reaction conditions for formation of the fluorophore-linked amic acids and their cyclization. After exploring several reaction conditions, eventually, we developed a two-step efficient procedure for the synthesis of fluorophore-linked maleimide derivatives **3a–e** in high yields. Among different solvents examined, high yields of amic acids **2a–f** were obtained in acetic acid with 1 equivalent of maleic anhydride and amines **1a–f** at room temperature. These amic acids were further smoothly cyclized into the corresponding maleimide derivatives **3a–e** in good to excellent yields by treatment with 1 equivalent of a Lewis acid and 1.5 equivalents of HMDS in refluxing benzene (Table). In order to improve the yield of fluoresceinylmaleimide **3f**, a



Scheme

Table. Synthesis of Fluorophore-Linked Maleamic Acids **2a–f** and Maleimides **3a–f**

Amine	Time (h)	Amic Acid	Yield (%)	Lewis Acid	Reflux Time (h)	Imide	Yield (%)
1a	1.5	**2a**	98	$ZnBr_2$	1.5	**3a**	98
1b	2.0	**2b**	94	$ZnCl_2$	1.0	**3b**	90
1c	2.0	**2c**	96	$ZnBr_2$	1.5	**3c**	97
1d	2.0	**2d**	96	$ZnBr_2$	1.5	**3d**	94
1e	2.5	**2e**	98	$ZnBr_2$	1.5	**3e**	86
1f	4.0	**2f**	92	$ZnCl_2$	2.5	**3f**	87

mixture of benzene and DMF (10%) was used as the solvent for the cyclization reaction of fluoresceinylmaleamic acid **2f**. Although the cyclization of amic acids **2a–e** proceeded well in toluene and also in xylene, the yields of maleimide derivatives **3a–e** were relatively better in benzene.

Among different Lewis acids examined, cyclization of fluorophore-linked maleamic acids proceeded well with 1 equivalent of $ZnBr_2$ under optimized reaction conditions. *N*-(1-Anthryl)maleimide **(3b)** and *N*-(5-fluoresceinyl)maleimide **(3f)** were obtained in better yields using $ZnCl_2$, since extensive side products were formed in the reaction with $ZnBr_2$. It is noteworthy that when amic acid cyclization was carried out with even a trace amount of unreacted amine impurities, yields of maleimides **3a–f** were reduced drastically and formation of unidentified products was observed after prolonged reflux. After completion of the reaction, fluorophore-linked maleimide derivatives, except anthrylmaleimide **3b** and fluoresceinylmaleimide **3f**, were obtained substantially pure (over 95% purity as shown by their NMR spectra) on simple aqueous workup of the reaction mixture, whereas anthrylmaleimide **3b** was obtained in 90% yield after silica gel column chromatography. Anthrylmaleimide derivatives are known[3e] to easily undergo a thermal Diels–Alder type of reaction, and a mixture of complex products was obtained on overheating the reaction mixture involving anthrylmaleamic acid **2b**. After the reaction with **2f**, pure fluoresceinylmaleimide **3f** was obtained in 87% yield[7] by precipitation from the aqueous phase at pH 6.0. In non-aqueous workup, the crude fluoresceinylmaleimide **3f** was obtained as the *O,O'*-bis(trimethylsilyl) ether in almost quantitative yield. However, due to its acid- and moisture-sensitive nature, our effort to isolate *N*-{5-[*O,O'*-bis(trimethylsilyl)fluoresceinyl]}maleimide in pure form was unsuccessful.

It is known that some maleimide derivatives, upon reacting with thiol compounds, instantaneously recover their high fluorescence.[8] Furthermore, we also confirmed that not only maleimide derivatives **3a–e** but also maleamic acids **2a–e** showed weak fluorescence compared with the parent, highly fluorescent amines **1a–e**. In contrast, fluoresceinylmaleamic acid **2f** and fluoresceinylmaleimide **3f** showed, as such, strong fluorescence compared with the parent, non-fluorescent fluoresceinamine[9] **1f**.

In summary, the present method provides a common and simple approach for the synthesis of various fluorophore-linked maleimide derivatives in a short reaction time with minimum purification efforts. Furthermore, this mild methodology has tolerance for the synthesis of various functional groups containing maleimide derivatives which can be used for a wide variety of applications. Due to the simplicity and general convenience this method can be adopted for the large-scale synthesis of different fluorophore-linked maleimide derivatives.

All fluorophore amines were purchased from Nacalai Tesque, except fluoresceinamine purchased from Aldrich Chemical Co., and used without further purification. Benzene was dried prior to use by distillation over CaH_2. HMDS was distilled before use. $ZnCl_2$ and $ZnBr_2$ were used after sublimation. Mps are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini-200 (200 MHz, 50 MHz) spectrometer. Chemical shifts are expressed in δppm with TMS as an internal standard.

Synthesis of Amic Acids 2a–f; General Procedure:
To a stirred solution of amine **1a–f** (0.5 mmol) in AcOH (50 mL) was added maleic anhydride (0.5 mmol) and the resulting solution was stirred at r.t. for the time specified in the Table. Precipitated amic acid **2a–f** was filtered, washed with EtOAc (100 mL), dried and used as such without further purification.

N-*(1-Pyrenyl)maleamic Acid* **(2a)**:[3d] yield: 98%; yellow solid; mp 181.5–182.5 °C.
IR (KBr): ν = 1710, 1530, 1460, 1410, 1300, 1260 cm^{-1}.
^1H NMR (DMSO-d_6): δ = 13.10–13.50 (br, 1H), 10.86 (s, 1H), 8.00–8.45 (m, 9H), 6.80 (d, *J* = 12.1 Hz, 1H), 6.39 (d, *J* = 12.1 Hz, 1H).
^{13}C NMR (DMSO-d_6): δ = 167.0, 164.7, 133.0, 131.0, 130.9, 130.6, 130.3, 128.8, 127.4, 127.0, 126.6, 125.5, 125.2, 125.1, 124.5, 124.3, 123.9, 123.5, 122.6.
MS: *m/z* (%) = 315 (M^+, 2), 297 (100), 243 (18), 227 (88), 217 (99) 189 (99).

N-*(1-Anthryl)maleamic Acid* **(2b)**:[3e] yield: 94%; yellow solid; mp 203.0–205.0 °C.
IR (KBr): ν = 1710, 1640, 1530, 1410, 1290 cm^{-1}.
^1H NMR (DMSO-d_6): δ = 13.35 (br, 1H), 10.63 (s, 1H), 8.79 (s, 1H), 8.60 (s, 1H), 8.00–8.20 (m, 2H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.77 (d, *J* = 7.1 Hz, 1H), 7.46–7.60 (m, 3H), 6.79 (d, *J* = 12.2 Hz, 1H), 6.38 (d, *J* = 12.2 Hz, 1H).
^{13}C NMR (DMSO-d_6): δ = 167.0, 164.5, 132.8, 131.9, 131.3, 131.1, 130.3, 128.5, 128.0, 126.6, 126.2, 126.1, 125.2, 121.7, 120.8.
MS: *m/z* (%) = 291 (M^+, 13), 273 (42), 246 (6), 193 (100), 165 (93).

N-*[4-Methylcoumarin-7-yl]maleamic Acid* **(2c)**: yield: 96%; yellow solid; mp 204.5–205.5 °C.
IR (KBr): ν = 1720, 1690, 1630, 1590, 1530, 1400, 1330 cm^{-1}.
^1H NMR (DMSO-d_6): δ = 12.60–13.20 (s, 1H), 10.68 (s, 1H), 7.75 (d, *J* = 1.8 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.51 (dd, *J* = 7.9, 1.8 Hz,

1H), 6.48 (d, *J* = 11.9 Hz, 1H), 6.32 (d, *J* = 11.9 Hz, 1H), 6.25 (s, 1H), 2.40 (s, 3H).
^{13}C NMR (DMSO-d_6): δ = 167.0, 163.9, 160.0, 153.7, 153.1, 142.1, 131.6, 130.5, 126.1, 115.5, 112.6, 106.0, 18.1.
MS: *m/z* (%) = 273 (M^+, 10), 255 (100), 227 (88), 175 (79), 147 (78), 119 (39).
Anal.: calcd for $C_{14}H_{11}NO_5$: C, 61.53; H, 4.05; N, 5.12. Found: C, 61.25; H, 4.10; N, 4.86.

N-*(2-Fluorenyl)maleamic Acid* **(2d)**:[10] yield: 96%; yellow solid; mp 271.0–273.0 °C.
IR (KBr): ν = 1700, 1630, 1530, 1470, 1420, 1280 cm^{-1}.
^{1}H NMR (DMSO-d_6): δ = 13.30 (br, 1H), 10.53 (s, 1H), 7.96 (s, 1H), 7.79 (m, 2H), 7.52–7.58 (m, 2H), 7.20–7.40 (m, 2H), 6.50 (d, *J* = 12.1 Hz, 1H), 6.29 (d, *J* =12.1 Hz, 1H), 3.90 (s, 2H).
^{13}C NMR (DMSO-d_6): δ = 166.9, 163.4, 144.0, 143.1, 141.0, 137.6, 137.3, 132.0, 130.9, 126.9, 126.4, 125.2, 120.3, 119.7, 118.6, 116.0, 36.7.
MS: *m/z* (%) = 279 (M^+, 12), 261 (51), 204 (6), 181 (100), 164 (16), 152 (28).

N-*(3-Fluoranthenyl)maleamic Acid* **(2e)**:[11] yield: 98%; yellow solid; mp 179.5–181.5 °C.
IR (KBr): ν = 1715, 1640, 1560, 1460 cm^{-1}.
^{1}H NMR (DMSO-d_6): δ = 13.32 (br, 1H), 10.69 (s, 1H), 8.27 (d, *J* = 8.3 Hz, 1H) 7.99–8.18 (m, 5H), 7.74 (t, *J* = 7.6 Hz, 1H), 7.30–7.45 (m, 2H), 6.76 (d, *J* = 12.0 Hz, 1H), 6.42 (d, *J* = 12.0 Hz, 1H).
^{13}C NMR (DMSO-d_6): δ = 167.2, 164.2, 138.8, 138.5, 136.3, 134.3, 133.0, 132.4, 132.1, 130.4, 128.0, 127.9, 127.4, 124.0, 123.1, 122.0, 121.7, 121.3, 121.1.
MS: *m/z* (%) = 315 (M^+, 1), 297 (34), 227 (5), 217 (100), 189 (26).

N-*(5-Fluoresceinyl)maleamic Acid* **(2f)**: yield: 92%; yellow solid; mp >300 °C.
IR (KBr): ν = 1710, 1680, 1590, 1540, 1470, 1310 cm^{-1}.
^{1}H NMR (DMSO-d_6): δ = 12.92 (br, 1H), 10.71 (s, 1H), 10.02 (s, 2H), 8.30 (s, 1H), 7.84 (dd, *J* = 8.3, 1.5 Hz, 1H), 7.18 (d, *J* = 8.3 Hz, 1H), 6.49–6.65 (m, 7H), 6.30 (d, *J* = 11.8 Hz, 1H).
^{13}C NMR (DMSO-d_6): δ = 168.6, 164.0, 159.6, 152.0, 147.4, 140.4, 132.3, 129.2, 127.15, 126.8, 124.7, 114.0, 112.7, 109.8, 102.3, 83.2.
MS (SI): *m/z* (%) = 446 (M + 1, 14), 289 (66), 273 (9), 260 (5), 246 (10).
Anal.: calcd for $C_{24}H_{15}NO_8$: C, 64.72; H, 3.39; N, 3.14. Found: C, 64.88; H, 3.47; N, 2.85.

Synthesis of Maleimide Derivatives 3a–e; General Procedure:
HMDS (0.15 mmol) was added to a stirred suspension of amic acid **2a–e** (0.1 mmol) and $ZnBr_2$ (0.1 mmol) in benzene (20 mL) at 65 °C. Immediately a clear solution was formed leaving thick paste at the bottom of the flask. The mixture was refluxed for the time specified in the Table and then, after cooling to r.t., it was poured into ice-water and extracted with EtOAc. The combined extracts were washed successively with 0.1 N HCl (30 mL) and brine, dried (Na_2SO_4) and evaporated to give pure (>95% as shown by NMR spectra) maleimide derivative **3a–e**.

N-*(1-Pyrenyl)maleimide* **(3a)**:[3d] yield: 98%; yellow needles; mp 239.0–240.0 °C.
IR (KBr): ν = 1710, 1510, 1450, 1400, 1150 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ = 8.00–8.32 (m, 7H), 7.70–7.90 (m, 2H), 7.05 (s, 2H).
^{13}C NMR (DMSO-d_6): δ = 170.9, 135.2, 131.3, 130.6, 130.3, 128.7, 128.4, 128.3, 127.3, 127.2, 126.9, 126.2, 126.0, 125.5, 125.2, 124.3, 123.5, 122.2.
MS: *m/z* (%) = 297 (M^+, 100), 269 (20), 243 (25), 227 (24), 214 (55).

N-*(1-Anthryl)maleimide* **(3b)**:[3e] $ZnCl_2$ was used as a Lewis acid; purified by chromatography on silica gel (EtOAc/hexane 1:9); yield: 90%; yellow needles; mp >300 °C.
IR (KBr): ν = 1720, 1470, 1400, 1150 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ = 8.53 (s, 1H), 7.90–8.20 (m, 4H), 7.32–7.60 (m, 4H), 7.05 (s, 2H).
^{13}C NMR (DMSO-d_6): δ = 170.8, 135.2, 131.8, 131.7, 131.5, 129.9, 128.4, 128.1, 127.3, 127.1, 126.3, 124.9, 121.5.
MS: *m/z* (%) = 273 (M^+, 100), 246 (5), 217 (21), 190 (29), 178 (24).

N-*[4-Methylcoumarin-7-yl]maleimide* **(3c)**: yield: 97%; yellow needles; mp 249.5–251.0 °C.
IR (KBr): ν = 1780, 1720, 1620, 1410, 1160 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ = 7.69 (d, *J* = 8.5 Hz, 1H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.40 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.91 (s, 2H), 6.33 (d, *J* = 1.2 Hz, 1H), 2.46 (d, *J* = 1.2 Hz, 3H).
^{13}C NMR (DMSO-d_6): δ = 169.5, 159.7, 152.9, 135.1, 134.6, 125.9, 122.2, 118.8, 114.6, 114.1, 18.2.
MS: *m/z* (%) = 255 (M^+, 100), 227 (78), 199 (8), 172 (10), 157 (7), 147 (4).
nal.: calcd for $C_{14}H_9NO_4$: C, 65.88; H, 3.55; N, 5.48. Found: C, 65.59; H, 3.77; N, 5.22.

N-*(2-Fluorenyl)maleimide* **(3d)**:[10] yield: 94%; yellow needles; mp 190.0–191.5 °C.
IR (KBr): ν = 1710, 1490, 1460, 1400, 1170 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ = 7.78-7.90 (m, 2H), 7.25–7.60 (m, 5H), 6.88 (s, 2H), 3.95 (s, 2H).
^{13}C NMR (DMSO-d_6): δ = 170.2, 143.7, 143.5, 140.7, 140.4, 134.8, 130.2, 127.2, 127.0, 125.7, 125.3, 123.9, 120.4, 120.2, 36.6.
MS: *m/z* (%) = 261 (M^+, 100), 233 (4), 204 (11), 190 (3), 178 (5), 164 (22), 83 (81).

N-*(3-Fluoranthenyl)maleimide* **(3e)**:[11] yield: 86%; pale yellow needles; mp 270.0–271.0 °C.
IR (KBr): ν = 1720, 1490, 1440, 1410, 1380, 1150 cm^{-1}.
^{1}H NMR ($CDCl_3$): δ = 7.84–7.99 (m, 4H), 7.58–7.67 (m, 1H), 7.36–7.52 (m, 4H), 6.99 (s, 2H).
^{13}C NMR (DMSO-d_6): δ = 170.7, 139.1, 139.0, 138.1, 137.2, 136.5, 135.1, 132.2, 129.2, 128.4, 128.2, 127.4, 123.4, 122.7, 122.4, 122.3, 121.5, 120.7.
MS: *m/z* (%) = 297 (M^+, 100), 269 (7), 241 (20), 227 (17), 214 (30).

***N*-(5-Fluoresceinyl)maleimide (3f):**
HMDS (0.324 mg, 2.0 mmol) was added to a stirred suspension of amic acid **2f** (222 mg, 0.5 mmol) and $ZnCl_2$ (136mg, 1.0 mmol) in a mixture of benzene (27 mL) and DMF (3 mL) and the resulting mixture was refluxed for 2.5 h. After cooling to r.t., the mixture was filtered and filtrate was concentrated under vacuum. The residual DMF portion was poured into ice-water (50 mL) and the aqueous phase was acidified to pH 6.0 by adding 0.1 N HCl. On cooling, fluoresceinylmaleimide **3f** (184 mg) was obtained in 87% yield as an orange-yellow solid; mp >300 °C.
IR (KBr) ν = 1720, 1600, 1470, 1400, 1390, 1300, 1210 cm^{-1}.
^{1}H NMR (DMSO-d_6): δ = 10.20 (s, 2H), 8.00 (d, *J* = 1.1Hz, 1H), 7.80 (dd, *J* = 1.6, 8.2 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.28 (s, 2H), 6.55–6.71 (m, 6H).
^{13}C NMR (DMSO-d_6): δ = 169.7, 168.1, 159.7, 152.0, 151.2, 135.0, 133.5, 133.3, 129.2, 126.9, 124.8, 122.1, 112.9, 109.3, 102.4, 83.5.
MS (SI): *m/z* (%) = 428 (M + 1, 16), 324 (10), 289 (22), 273 (11).
Anal.: calcd for $C_{24}H_{13}NO_7$: C, 67.45; H, 3.06; N, 3.27. Found: C, 67.10; H, 2.89; N, 3.27.

P. Y. Reddy would like to thank Aisin Cosmos R&D, Kariya, Nagoya, Japan for financial support.

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