N-(Fmoc-*α*-aminoacyl)benzotriazoles: versatile synthetic reagents from proteinogenic amino acids

Alan R. Katritzky,* Anamika Singh, Danniebelle N. Haase, and Megumi Yoshioka

Center for Heterocyclic Compounds, University of Florida, Department of Chemistry, Gainesville, Florida 32611-7200, USA
E-mail: katritzky@chem.ufl.edu

Dedicated to Prof. Nicolo Vivona on the occasion of his 70th anniversary

Abstract

N-Fmoc-*α*-amino acids were smoothly converted into stable, crystalline *N*-(Fmoc-*α*-aminoacyl)benzotriazoles **2a-r** (69-90%). Compounds **2b,g** reacted with the chiral derivatizing reagent, (+/-)*α*-methylbenzylamine (**5** or **6**), to afford *α*-(*N*-Fmoc-amino)acid amides **3a,b** and **4a,b** (average yield 72 %) with no detectable racemization.

Keywords: *N*-(Fmoc-*α*-aminoacyl)benzotriazoles, *N*-Fmoc-*α*-amino acids, *α*-aminoacylation, *α*-(*N*-Fmoc-amino)acid amides

Introduction

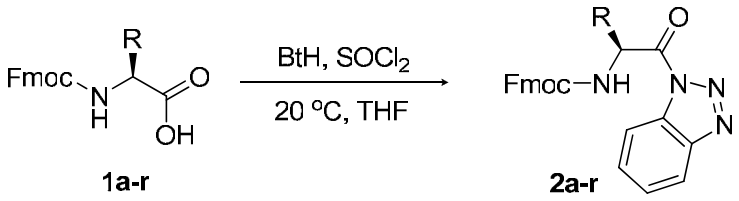
N-Protected α-amino acids need activation of the carboxylic acid function to allow peptide bond formation. Activation methods can be *in situ* with no isolable intermediates as exemplified by the use of carbodiimides, EDC, DCC and DIC, in combination with additives such as HOBt and HOAt.[1]

N-Acylbenzotriazoles have been utilized in (i) *N*-acylation for the preparation of primary, secondary and tertiary amides[2a-c] and *N*-acylsulfonamides,[2d] (ii) S-acylation for the synthesis of thiol esters[3] and (iii) C-acylation for the preparation of ketones,[4a] diketones,[4b] *β*-ketosulfones,[4c] enaminones[4d] and C-acylated pyrroles, indoles,[4e] 2-methylfurans and thiophenes[4f]. We have previously prepared the *N*-(Boc-, Fmoc- and Cbz-*α*-aminoacyl)benzotriazoles of some amino acids.[5a-c] We now report stable, crystalline *N*-(Fmoc-*α*-aminoacyl)benzotriazoles derivatives of 18 proteinogenic amino acids and their utilization in the synthesis of chiral *α*-(*N*-Fmoc-amino)acid amides.

Results and Discussion

Preparation of *N*-(Fmoc-*α*-aminoacyl)benzotriazoles 2a-r

Eighteen of the twenty natural, *N*-Fmoc-*α*-amino acids **1a-r** (purchased from Peptides International, Louisville, KY, USA and used without further purification) when treated with 1*H*-benzotriazole and thionyl chloride in THF at 20 °C for 2 hours[5a] (Scheme 1, Table 1), afforded crystalline *N*-(Fmoc-*α*-aminoacyl)benzotriazoles **2a-r** in 69-90% yields. Novel **2b-l**, **q-r** were characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis and high resolution mass spectrometry; known **2a** and **m-p** were verified by comparison of the melting points and spectroscopic data with that of the literature. The spectra of **2b-l**, **q-r** displayed the expected ^{13}C NMR chemical shifts at ca. δ 131, 127, 120, and 114 ppm, and that of the amide and carbamate carbonyl carbons at ca. δ 170 and 155 ppm, respectively.[5b]



Scheme 1. Preparation of *N*-(Fmoc-*α*-aminoacyl)benzotriazoles **2a-r** from the corresponding *N*-protected *α*-amino acids **1a-r**.

The *N*-(Fmoc-*α*-aminoacyl)benzotriazoles of arginine and aspargine were not obtained under these conditions; they appeared to be formed but were rapidly hydrolyzed before they could be isolated.

Preparation of *α*-(*N*-Fmoc-amino)acid amides 3a,b and 4a,b

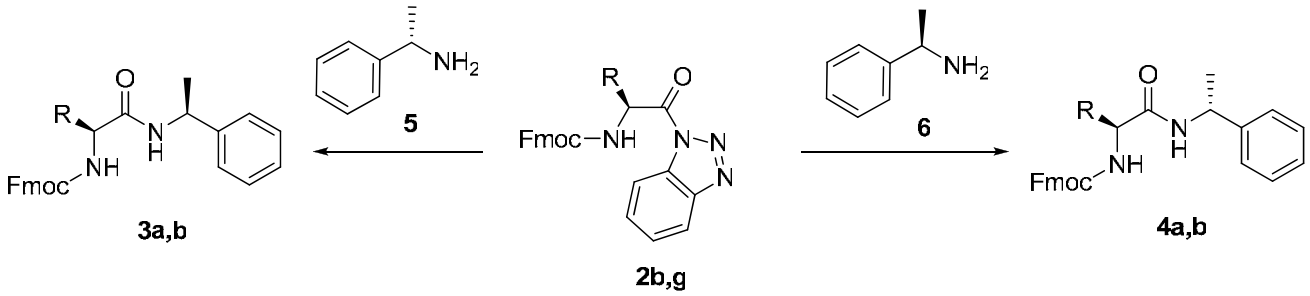
α-Methylbenzylamides of *N*-protected amino acids provide criteria for optical purity and stability towards racemization. *N*-(Fmoc-*α*-aminoacyl)benzotriazoles **2b**,**g** were separately reacted with L-*α*-methylbenzylamine **5** and D-*α*-methylbenzylamine **6** in THF at 20 °C to afford amides **3a,b** and **4a,b** in 66-74 % yields (average 76 %, Scheme 2, Experimental Section).

The diastereomeric excess (*de*) for the *α*-(*N*-Fmoc-amino)acid amides **3a,b** and **4a,b** were determined by ^{1}H NMR and HPLC. A comparison of the ^{1}H NMR spectra obtained from the derivatization of **2b** with D/L- and L-*α*-methylbenzylamine respectively demonstrated the chirality of **3a** and thus the conversion of the **1b** to **2b** also occurred with retention of chirality. HPLC analyses of **3a**, **4a** and corresponding diastereomeric mixtures provided further evidence for the smooth conversion of chiral **1b**,**g** to chiral **2b**,**g** (Table 2).

Table 1. Conversions of the 18 *N*-Fmoc-α-amino acids into *N*-(Fmoc-α-aminoacyl) benzotriazoles

Reactant	Product	Yield[a] (%)	mp (°C) (Lit. mp)	$[\alpha]_D$
Fmoc-L-Ile-OH **(1a)**	Fmoc-L-Ile-Bt **(2a)**	78	165.4-167.2[5c] (168.8-170.0)	-32.8
Fmoc-L-Val-OH **(1b)**	Fmoc-L-Val-Bt **(2b)**	84	148.3-149.8	-40.4
Fmoc-L-Thr(*t*Bu)-OH **(1c)**	Fmoc-L-Thr(*t*Bu)-Bt **(2c)**	80	62.2-65.0	-30.0
Fmoc-L-Lys(Boc)-OH **(1d)**	Fmoc-L-Lys(Boc)-Bt **(2d)**	75	138.4-140.6	-33.3
Fmoc-L-Glu(OtBu)-OH **(1e)**	Fmoc-L-Glu(OtBu)-Bt **(2e)**	81	65.5-67.6	-21.2
Fmoc-L-Ser(tBu)-OH **(1f)**	Fmoc-L-Ser(*t*Bu)-Bt **(2f)**	70	91.7-92.4	-14.8
Fmoc-L-Tyr(tBu)-OH **(1g)**	Fmoc-L-Tyr(tBu)-Bt **(2g)**	83	138.4-139.3	+15.0
Fmoc-L-Gln(Trt)-OH **(1h)**	Fmoc-L-Gln(Trt)-Bt **(2h)**	69	167.0-168.0	-16.3
Fmoc-L-Asp(OtBu)-OH **(1i)**	Fmoc-L-Asp(OtBu)-Bt **(2i)**	73	102.0-104.0	-11.1
Fmoc-L-Cys(Trt)-OH **(1j)**	Fmoc-L-Cys(Trt)-Bt **(2j)**	88	96.0-98.0	-11.0
Fmoc-L-His(Trt)-OH **(1k)**	Fmoc-L-His(Trt)-Bt **(2k)**	73	137.4-139.5	+13.0
Fmoc-L-Leu-OH **(1l)**	Fmoc-L-Leu-Bt **(2l)**	80	121.3-123.2	+53.1
Fmoc-L-Trp-OH **(1m)**	Fmoc-L-Trp-Bt **(2m)**	90	92.5-93.6[5c]; 192.4-195.2[b] (88.0-90.0)[5e]	+9.0
Fmoc-L-Phe-OH **(1n)**	Fmoc-L-Phe-Bt **(2n)**	85	159.1-160.2[5c] (136.5-137.4)[5d]	+3.4
Fmoc-L-Met-OH **(1o)**	Fmoc-L-Met-Bt **(2o)**	82	122.7-123.3[5c] (98.0-100.0)[5e]	-44.7
Fmoc-L-Ala-OH **(1p)**	Fmoc-L-Ala-Bt **(2p)**	72	160.0-160.3[5c] (160.7-161.3)[5d]	-60.8
Fmoc-L-Pro-OH **(1q)**	Fmoc-L-Pro-Bt **(2q)**	89	163.5-165.4[5c]	-60.5
Fmoc-Gly-OH **(1r)**	Fmoc-Gly-Bt **(2r)**	88	161.5-161.9[5c]	Non-chiral

[a] Isolated yield. [b]mp of polymorph.



For structural designation of R in **2b,g, 3a,b** and **4a,b** see Table 2

Scheme 2. Preparation of *N*-(acylamino)amides **3a,b** and **4a,b** from *N*-(Fmoc-*α*-aminoacyl) benzotriazoles **2b,g** and L- or D-PhCH(Me)NH_2 (**5** or **6**).

HOBt-based aminium- and phosphonium derivatives (PyBOP, HBTU, HATU, etc),[1] *N*-hydroxysuccinimide esters,[6] and *p*-nitrophenyl esters[7] are widely used in peptide synthesis but the preparative routes require multiple steps. *N*-(Fmoc-*α*-aminoacyl)benzotriazoles are easily prepared peptide coupling reagents whose generality has been demonstrated in the solution phase syntheses of sterically hindered peptides[8] or peptoids[9] and the solid phase preparation of simple oligopeptides[5c]. Additionally, *N*-(Fmoc-*α*-aminoacyl)benzotriazoles are fully amenable to microwave assisted syntheses.[5c, 8]

Table 2. Preparation of *N*-(acylamino)amides **3a,b** and **4a,b** from *N*-(Fmoc-*α*-aminoacyl) benzotriazoles **2b,g** and L- or D-PhCH(Me)NH_2 (**5** or **6**)

Reactant	Product	Yield[a] (%)	Retention time (min)
Fmoc-L-Val-Bt **(2b)**	Fmoc-L-Val-L-NHCH(Me)Ph **(3a)**	73	5.42
Fmoc-L-Val-Bt **(2b)**	Fmoc-L-Val-D/L-NHCH(Me)Ph **(3a+4a)**	74	5.41, 8.00
Fmoc-L-Tyr(tBu)-Bt **(2g)**	Fmoc-L-Tyr(*t*Bu)-D-NHCH(Me)Ph **(3b)**	74	1.91
Fmoc-L-Tyr(tBu)-Bt **(2g)**	Fmoc-L-Tyr(*t*Bu)-D/L-NHCH(Me)Ph **(3b+4b)**	66	1.73, 1.91

[a] Isolated yield. [b] For conditions, see the experimental section.

In summary, we describe the convenient, cost effective preparation of *N*-(Fmoc-*α*-aminoacyl)benzotriazoles **2a-r** (69-90%) storable at 20 °C for months without special handling. ^{1}H NMR and HPLC analyses of *N*-(Fmoc-*α*-aminoacyl)amides **3a,b** and **4a,b**, easily prepared in high yields, demonstrated that the chirality is maintained during amide bond formation.

Experimental Section

General Procedures. Reagents were obtained as follows: *N*-Fmoc-L-amino acids from Peptides International, Louisville, KY, USA; 1*H*-benzotriazole, dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate (EtOAc), hexanes, magnesium sulfate ($MgSO_4$) and sodium carbonate (Na_2CO_3) from Fischer Scientific, Fair Lawn, NJ, USA. Melting points were determined on a hot-stage apparatus and are uncorrected. ^{1}H (300 MHz, with TMS as the internal standard) and ^{13}C (75 MHz) NMR spectra were recorded in $CDCl_3$. Optical rotations were recorded on Perkin Elmer 241 polarimeter. HPLC analyses were performed on a Shimadzu instrument using a Zorbax Rx-C18 reverse phase column (4.6 x 150 mm) with UV detection at 210 nm, a flow rate of 1.0 mL/min and MeOH:H_2O as the eluting solvent. High resolution mass spectrometry was performed in the ESI (electrospray ionization) mode on an Agilent 6210 LC-TOF (liquid chromatography-time of flight) instrument. Elemental analysis was carried out in an Eager 200 CHN analyzer.

General procedure for the preparation of 2b-l, q, r

Thionyl chloride (5 mmol) was added dropwise to a solution of 1*H*-benzotriazole (20 mmol) in THF (50 mL). After stirring at room temperature for 30 min, *N*-Fmoc-amino acid (5 mmol) was added in one portion. After stirring for 2 h at room temperature, the solvent was evaporated in vacuo. The crude mixture obtained was dissolved in EtOAc (30 mL) and the organic layer washed with saturated Na_2CO_3 solution (30 mL x 3) and dried over $MgSO_4$. Concentration under reduced pressure gave the desired product, which was precipitated from dichloromethane-hexanes.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-3-methyl-1-oxobutan-2-ylcarbamate (Fmoc-L-Val-Bt, 2b).** White microcrystals (84%); mp 148.3-149.8 ^{o}C; $[\alpha]^{24}_D$ = -40.4^o (*c* = 1.5, $CHCl_3$); ^{1}H NMR δ 8.28 (d, *J* = 8.1 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 7.76 (d, *J* = 7.5 Hz, 2H), 7.69 (t, *J* = 7.8 Hz, 1H) 7.61 (d, *J* = 7.2 Hz, 2H), 7.54 (t, *J* = 7.5 Hz, 1H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (t, *J* = 7.2 Hz, 2H), 5.6 (dd, *J* = 9.5, 5.0 Hz, 1H), 5.63 (d, *J* = 9.3 Hz, 1H), 4.44 (d, *J* = 6.3 Hz, 2H), 4.24 (t, *J* = 7.2, 6.3 Hz, 1H), 2.58-2.42 (m, 1H), 1.13 (d, *J* = 6.6 Hz, 3H), 0.99 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR δ 172.0, 156.7, 146.4, 144.2, 144.1, 141.7, 131.4, 131.1, 128.1, 127.5, 127.0, 125.4, 120.8, 120.4, 114.8, 67.6, 59.8, 47.6, 32.1, 20.1, 17.5. Anal. Calcd for $C_{26}H_{24}N_4O_3$: C, 70.89; H, 5.49; N, 12.72; Found: C, 71.25; H, 5.57; N, 12.82.

***S*-(9*H*-Fluoren-9-yl)methyl(2*S*,3*R*) 1-(1*H*-benzotriazol-1-yl)-3-*tert*-butoxy-1-oxobutan-2-ylcarbamate (Fmoc-L-Thr(tBu)-Bt, 2c).** White microcrystals (80%); mp 62.2-65.0 ^{o}C; $[\alpha]^{24}_D$ = -30.0^o (*c* = 1.5, $CHCl_3$); ^{1}H NMR δ 8.28 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.78 (d, *J* = 7.5 Hz, 2H) 7.72-7.64 (m, 3H), 7.54 (t, J = 7.8 Hz, 1H), 7.45-7.31 (m, 4H), 5.94 (d, *J* = 9.6 Hz, 1H), 5.67 (dd, *J* = 9.6, 1.5 Hz, 1H), 4.62-4.51 (m, 1H), 4.43 (t, *J* = 6.6 Hz, 2H), 4.30 (t, *J* = 7.2 Hz, 1H), 1.43 (d, *J* = 6.0 Hz, 3H), 0.92 (s, 9H); ^{13}C NMR δ169.9, 156.8, 145.8, 143.9, 143.7, 141.3, 131.1, 130.8, 127.7, 127.1, 126.5, 125.2, 125.2, 120.3, 120.0, 114.2, 74.3, 68.0, 67.4, 60.6, 47.1, 28.0, 27.8,

21.1. Anal. Calcd for $C_{29}H_{30}N_4O_4$: C, 69.86; H, 6.06; N, 11.24; Found: C, 70.04; H, 6.23; N, 11.14.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-6-(*tert*-butoxycarbonylamino)-1-oxohexan-2-ylcarbamate (Fmoc-L-Lys(Boc)-Bt, 2d).** White microcrystals (75%); mp 138.4-140.6 °C; $[\alpha]^{24}_D$ = -30.0° (*c* = 1.5, $CHCl_3$); ^{1}H NMR δ 8.27 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.5 Hz, 2H), 7.68 (overlapped t, *J* = 7.5 Hz, 1H), 7.65-7.60 (m, 2H) 7.54 (t, *J* = 7.8 Hz, 1H), 7.40 (t, *J* = 7.1 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 5.87 (d, *J* = 7.8 Hz, 1H), 5.82-5.72 (m, 1H), 4.62 (br s, 1H), 4.49-4.37 (m, 2H), 4.24 (t, *J* = 6.9 Hz, 1H), 3.20-3.00 (m, 2H), 2.20-1.90 (m, 2H), 1.60-1.50 (m, 4H), 1.43 (s, 9H); ^{13}C NMR δ 171.7, 156.2, 146.0, 143.8, 143.6, 141.2, 131.1, 130.7, 127.7, 127.1, 126.5, 125.1, 120.3, 120.0,114.4, 79.3, 67.2, 54.5, 47.1, 39.6, 32.2, 29.6, 28.4, 22.5. Anal. Calcd for $C_{32}H_{35}N_5O_5$: C, 67.47; H, 6.19; N, 12.29; Found: C, 67.38; H, 6.22; N, 11.90.

S-*tert*-Butyl 4-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-5-(1*H*-benzotriazol-1-yl)-5-oxopentanoate (Fmoc-L-Glu(OtBu)-Bt, 2e). White microcrystals (81%); mp 65.5-67.6 °C; $[\alpha]^{20}_D$ -21.2° (*c* = 2.4, $CHCl_3$); ^{1}H NMR δ 8.19 (d, *J* = 8.1 Hz, 1H), 8.08 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 7.5 Hz, 2H), 7.65-7.39 (m, 4H), 7.33 (t, *J* = 6.9 Hz, 2H), 7.24 (t, *J* = 7.2 Hz, 2H), 7.00 (br s, 1H), 5.91 (d, *J* = 8.1 Hz, 1H), 5.78-5.68 (m, 1H), 4.42-4.27 (m, 2H), 4.16 (t, *J* = 6.9 Hz, 1H), 2.41-2.28 (m, 2H), 2.25-2.10 (m, 2H), 1.36 (s, 9H); ^{13}C NMR δ 172.0, 171.2, 156.1, 146.0, 143.8, 143.6, 141.2, 131.1, 130.8, 127.7, 127.0, 126.6, 125.1, 120.3, 119.9, 114.4, 81.2, 67.2, 54.5, 47.1, 31.6, 28.0, 27.5. HRMS calcd for $C_{30}H_{30}N_4O_5$ $[M+Na]^+$ 549.2108, found 549.2071.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-3-*tert*-butoxy-1-oxopropan-2-ylcarbamate (Fmoc-L-Ser(tBu)-Bt, 2f).** White microcrystals (70%); mp 91.7-92.4°C; $[\alpha]^{20}_D$ -14.8° (*c* = 2.4, $CHCl_3$); ^{1}H NMR δ 8.30 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.72-7.63 (m, 2H), 7.58-7.52 (m, 2H), 7.44-7.26 (m, 4H), 6.02 (d, *J* = 9.0 Hz, 1H), 5.88-5.86 (m, 1H), 4.47-4.35 (m, 2H), 4.31-4.22 (m, 2H), 3.92 (dd, *J* = 9.0, 3.2 Hz, 1H), 1.03 (s, 9H); ^{13}C NMR δ 169.5, 156.2, 143.9, 143.7, 141.3, 131.2, 131.0, 127.8, 127.1, 126.5, 126.1, 125.2, 120.3, 120.0, 114.4, 74.0, 67.5, 62.9, 55.9, 47.1, 27.1. HRMS calcd for $C_{28}H_{28}N_4O_4$ $[M+Na]^+$ 507.2003, found 507.1986.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-3-(4-*tert*-butoxyphenyl)-1-oxopropan-2-ylcarbamate (Fmoc-L-Tyr(tBu)-Bt, 2g).** White microcrystals (83%); mp 138.4-139.3°C; $[\alpha]^{20}_D$ +15.0° (*c* = 1.9, $CHCl_3$); ^{1}H NMR δ 8.19 (d, *J* = 8.4 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.62 (t, *J* = 7.5 Hz, 1H), 7.55 (t, *J* = 4.5 Hz, 2H), 7.48 (t, *J* = 7.5 Hz, 1H), 7.38-7.23 (m, 4H), 7.03 (d, *J* = 8.1 Hz, 2H), 6.83 (d, *J* = 7.8 Hz, 2H), 6.13-6.06 (m, 1H), 5.83 (d, *J* = 8.4 Hz, 1H), 4.38 (t, *J* = 6.6 Hz, 2H), 4.20-4.16 (m, 1H), 3.39 (dd, *J* = 13.5, 5.4 Hz, 1H), 3.21 (dd, *J* = 13.5, 8.0 Hz, 1H), 1.24 (s, 9H); ^{13}C NMR δ 170.9, 155.7, 154.4, 145.8, 143.6, 143.5, 141.1, 130.8, 130.6, 129.7, 129.6, 127.6, 126.9, 126.4, 124.9, 124.2, 120.2, 119.8, 114.1, 78.4, 67.1, 55.6, 46.9, 38.2, 28.6. Anal. Calcd for $C_{34}H_{32}N_4O_4$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.84; H, 6.00; N, 9.70.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-1,5-dioxo-5-(tritylamino)pentan-2-ylcarbamate (Fmoc-L-Gln(Trt)-Bt, 2h).** White microcrystals (69%); mp 167.0-168.0 °C; $[\alpha]^{20}_{D}$ -16.3° (*c* = 1.4, $CHCl_3$); ^{1}H NMR δ 8.26 (d, *J* = 8.4 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 7.75 (d, *J* = 6.9 Hz, 2H), 7.68 (t, *J* = 7.2 Hz, 1H), 7.59-7.51 (m, 2H), 7.44-7.38 (m, 2H), 7.32-7.26 (m, 12H), 7.23-7.20 (m, 6H), 6.81 (s, 1H), 6.14 (d, *J* = 7.2 Hz, 1H), 5.83-5.22 (m, 1H), 4.52-4.45 (m, 1H), 4.36 (t , *J* = 6.6 Hz, 1H), 4.23 (t, *J* = 6.3 Hz, 1H), 2.57 (t, *J* = 6.6 Hz, 2H), 2.44 (br s 1H), 2.27 (br s 1H); ^{13}C NMR δ 171.1, 170.8, 156.5, 146.1, 144.5, 143.9, 143.7, 141.3, 131.1, 130.8, 128.7, 128.1, 127.8, 127.2, 126.6, 125.2, 120.4, 120.0, 114.5, 70.9, 67.3, 54.7, 47.2, 35.6, 27.7. Anal. Calcd for $C_{45}H_{37}N_5O_4$: C, 75.93; H, 5.24; N, 9.84. Found: C, 75.82; H, 5.46; N, 9.89.

***S*-*tert*-Butyl 3-(((9*H*-fluoren-9-yl)methoxy)carbonylamino)-4-(1*H*-benzotriazol-1-yl)-4-oxobutanoate (Fmoc-L-Asp(O*t*Bu)-Bt, 2i).** White microcrystals (73%); mp 102.0-104.0 °C; $[\alpha]^{20}_{D}$ -11.1° (*c* = 2.5, $CHCl_3$); ^{1}H NMR δ 8.29 (d, *J* = 8.1 Hz, 1H), 8.15 (d, *J* = 8.1 Hz, 1H), 7.75 (d, *J* = 7.2 Hz, 2H), 7.68 (t, *J* = 7.5 Hz, 1H), 7.60-7.51 (m, 3H), 7.42-7.28 (m, 4H), 6.15 (d, *J* = 6.9 Hz, 1H), 6.00-5.86 (m 1H), 4.44-4.40 (m, 2H), 4.27-4.25 (m, 1H), 3.26 (dd, *J* = 15.5, 5.6 Hz, 1H), 3.14 (dd, *J* = 18.6, 5.4 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR δ 169.5, 169.1, 155.7, 145.9, 143.7, 143.5, 141.2, 131.1, 131.8, 127.7, 127.0, 126.6, 125.1, 120.3, 120.0, 114.3, 82.3, 67.3, 51.9, 47.0, 38.5, 27.9. Anal. Calcd for $C_{29}H_{28}N_4O_5$: C, 67.96; H, 5.51; N, 10.93. Found: C, 67.98; H, 5.81; N, 10.96.

***R*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-1-oxo-3-(tritylthio)-propan-2-ylcarbamate (Fmoc-L-Cys(Trt)-Bt, 2j).** White microcrystals (88%); mp 96.0-98.0°C; $[\alpha]^{20}_{D}$ -11.0° (*c* = 2.0, $CHCl_3$); ^{1}H NMR δ 8.24 (d, *J* = 8.1 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 7.78-7.76 (m, 1H), 7.70 (t, *J* = 7.5 Hz, 1H), 7.64-7.56 (m, 3H), 7.41-7.38 (m, 2H), 7.33-7.30 (m, 7H), 7.19-7.11 (m, 11H), 5.78-5.70 (m, 1H), 5.52 (d, *J* = 7.2 Hz, 1H), 4.41 (d, *J* = 6.9 Hz, 2H), 4.24 (t, *J* = 6.9 Hz, 1H), 3.13. (dd, *J* = 14.1, 5.7 Hz, 1H), 2.92 (dd, *J* = 12.6, 6.2 Hz, 1H); ^{13}C NMR δ 169.1, 155.6, 146.0, 144.0, 143.5, 141.3, 131.1, 130.8, 129.4, 128.0, 127.8, 127.1, 127.0, 126.7, 125.2, 120.4, 120.0, 114.5, 67.5, 67.3, 53.9, 47.1, 34.1. Anal. Calcd for $C_{43}H_{34}N_4O_3S$: C, 75.20; H, 4.99; N, 8.16. Found: C, 75.09; H, 5.28; N, 7.82.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-1-oxo-3-(1-trityl-1*H*-imidazol-4-yl)propan-2-ylcarbamate (Fmoc-L-His(Trt)-Bt, 2k).** Yellow microcrystals (73%); mp 137.4-139.5°C; $[\alpha]^{24}_{D}$ = -60.5° (*c* = 1.5, $CHCl_3$); ^{1}H NMR (300 Hz, $CDCl_3$) δ 8.19 (d, *J* = 8.1 Hz, 1H), 8.07 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 7.5 Hz, 2H), 7.66-7.57 (m, 3H), 7.42-7.44 (m, 3H), 7.38-7.31 (m, 4H), 7.26-7.24 (m, 15H), 6.45 (s, 1H), 6.08-5.80 (m, 1H), 4.40-4.20 (m, 3H), 3.50-3.38 (m, 2H); ^{13}C NMR (75Hz, $CDCl_3$) δ 170.5, 156.3, 145.8, 143.8, 142.0, 141.1, 139.0, 135.5, 130.5, 129.6, 129.6, 128.0, 127.6, 127.0, 126.3, 125.2, 120.2, 119.8, 114.3, 103.3, 77.2, 70.0, 60.4, 55.4, 30.3. HRMS calcd for $C_{46}H_{36}N_6O_3$ $[M+H]^+$ 721.2922, found 721.2919.

***S*-(9*H*-Fluoren-9-yl)methyl 1-(1*H*-benzotriazol-1-yl)-4-methyl-1-oxopentan-2-ylcarbamate (Fmoc-L-Leu-Bt, 2l).** White microcrystals (80%); mp 121.3-123.2 °C; $[\alpha]^{24}_{D}$ = + 53.1° (*c* = 1.5, DMF); ^{1}H NMR δ 8.27 (d, *J* = 8.2 Hz, 1H), 8.16 (d, *J* = 8.1 Hz, 1H), 7.77 (d, *J* = 7.3 Hz, 2H), 7.69 (overlapped t, *J* = 7.1 Hz, 1H), 7.62-7.40 (m, 2H), 7.54 (overlapped t, *J* = 7.8 Hz, 1H), 7.41 (t, *J* = 7.0 Hz, 2H), 7.32 (t, *J* = 7.1 Hz, 2H), 5.85 (t, *J* = 7.8 Hz, 1H), 5.54-5.44 (m, 1H), 4.45 (d,

J = 7.0 Hz, 2H), 4.25 (t, *J* = 6.6 Hz, 1H), 1.88 (m, 2H), 1.82-1.71 (m, 1H), 1.11 (d, *J* = 4.9 Hz, 3H), 0.99 (d, *J* = 5.4 Hz, 3H); ^{13}C NMR δ 172.4, 156.1, 146.0, 143.8, 143.6, 141.3, 131.1, 130.7, 127.7, 127.0, 126.5, 125.0, 120.3, 120.0, 114.4, 67.1, 53.0, 47.1, 41.9, 25.2, 23.2, 21.3. Anal. Calcd for $C_{27}H_{26}N_4O_3$: C, 71.35; H, 5.77; N, 12.33; Found: C, 71.19; H, 6.06; N, 12.21.

***S*-(9*H*-Fluoren-9-yl)methyl 2-(1*H*-benzotriazole-1-carbonyl)pyrrolidine-1-carboxylate (Fmoc-L-Pro-Bt, *ca.* 1:1 mixture of rotamers, 2q).** White microcrystals (89%); mp 163.5-165.4 °C; $[\alpha]^{24}_D$ = -60.5° (*c* = 1.5, DMF); ^{1}H NMR δ 8.29 (d, *J* = 8.2 Hz, 0.5H), 8.20 (d, *J* = 8.2 Hz, 0.5H), 8.14 (d, *J* = 8.1 Hz, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.74-7.28 (m, 6H), 7.21 (t, *J* = 6.0 Hz, 1.5H), 7.09 (t, *J* = 6.7 Hz, 0.5H), 6.89-6.78 (m, 1H), 5.89 (d, *J* = 4.2 Hz, 0.5H), 5.86 (d, *J* = 4.2 Hz, 0.5H), 5.44 (d, *J* = 3.3 Hz, 0.5H), 5.41 (d, *J* = 3.9 Hz, 0.5H), 4.61-4.53 (m, 1H), 4.52-4.43 (m, 0.5H), 4.40-4.26 (m, 0.5H), 4.02 (t, *J* = 5.0 Hz, 0.5H), 3. 90-3.81 (m, 0.5H), 3.77-3.57 (m, 1.5H), 2.71-2.57 (m, 0.5H), 2.56-2.42 (m, 0.5H), 2.31-2.19 (m, 1.5H), 2.18-2.00 (m, 1H), 1.99-1.88 (m, 1.5H); ^{13}C NMR δ 171.0, 170.6, 154.9, 154.1, 146.0, 144.0, 143.8, 143.5, 141.3, 141.0, 140.8, 131.2, 131.2, 130.5, 130.5, 127.7, 127.4, 127.1, 126.9, 126.8, 126.5, 126.4, 126.4, 125.2, 125.1, 124.1, 124.0, 120.2, 120.2, 120.0, 119.7, 119.4, 114.6, 114.5, 67.7, 66.5, 60.0, 59.2, 47.2, 47.0, 46.9, 31.6, 30.7, 24.5, 23.2. Anal. Calcd for $C_{26}H_{22}N_4O_3$: C, 71.22; H, 5.06; N, 12.78; Found: C, 71.16; H, 5.03; N, 13.12.

(9*H*-Fluoren-9-yl)methyl 2-(1*H*-benzotriazol-1-yl)-2-oxoethylcarbamate (Fmoc-Gly-Bt, 2r). White microcrystals (88%); mp 161.5-161.9 °C; ^{1}H NMR δ 8.25 (d, *J* = 8.2 Hz, 1H), 8.15 (d, *J* = 8.4Hz, 1H), 7.77 (d, *J* = 7.4Hz, 2H), 7.71-7.68 (overlapped t, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 7.54 (t , *J* = 7.7 Hz, 2H), 7.41 (t, *J* = 7.2 Hz, 1H), 7.33 (t, *J* = 7.2 Hz, 2H), 7.14 (br s, 1H), 5.59 (t, *J* = 5.4 Hz, 1H), 5.10 (d, *J* = 5.7 Hz, 2H), 4.49 (d, *J* = 6.9 Hz, 2H), 4.28 (t, *J* = 6.9 Hz, 1H); ^{13}C NMR δ 168.4, 156.5, 146.0, 143.7, 141.3, 130.9, 130.8, 127.7, 127.1, 126.6, 125.1, 120.4, 120.0, 114.0, 67.4, 47.0, 44.8. Anal. Calcd for $C_{23}H_{18}N_4O_3$: C, 69.34; H, 4.55; N, 14.03; Found: C, 69.40; H, 4.36; N, 14.08.

General procedure for the preparation of 3a,b, 4a,b, (3a+4a) and (3b+4b)

N-(Fmoc-*α*-aminoacyl)benzotriazoles **2b,g** (1 mmol) was dissolved in THF and L-*α*-methylbenzylamine **5**, D-*α*-methylbenzylamine **6** or *α*-methylbenzylamine (**5+6**) (1 mmol) was added to the solution. The mixture was stirred at room temperature and monitored by TLC. On completion of the reaction the solvent was evaporated in vacuo. The resulting solid was dissolved in EtOAc (30 mL) and washed with saturated Na_2CO_3 (30 mL x 3) and dried with $MgSO_4$. The solution was reduced to dryness *in vacuo* to yield **3a,b**, **4a,b,** (**3a+4a**) and (**3b+4b**).

(9*H*-Fluoren-9-yl)methyl *S*-3-methyl-1-oxo-1-((*R*)-1-phenylethylamino)butan-2-ylcarbamate (3a). White powder (73%); mp 205.7-206.2 °C; $[\alpha]^{25}_D$ = -32.6° (*c* = 2.4, $CHCl_3$); ^{1}H NMR δ 7.82 (d, *J* = 7.8 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.45 (t, *J* = 7.2 Hz, 2H), 7.38-7.32 (m, 7H), 6.34 (d, *J* = 6.3 Hz, 1H), 5.57 (d, *J* = 8.1 Hz, 1H), 5.17 (quintet, *J* = 7.2 Hz, 15.6 Hz, 1H), 4.50-4.35 (m, 2H), 4.28-4.20 (m, 1H), 4.01 (t, *J* = 5.6 Hz, 1H), 2.18-2.06 (m, 1H), 1.75 (s, 1H), 1.52 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR 170.3, 156.5, 143.7, 142.8, 141.3, 128.6, 127.7, 127.4,

127.1, 126.1, 125.0, 120.0, 67.0, 60.5, 48.9, 47.1, 31.3, 21.7, 19.2, 17.9. Anal. Calcd for $C_{28}H_{30}N_2O_3$: C, 75.99; H, 6.83; N, 6.33; Found: C, 75.90; H, 6.99; N, 6.09.

(9*H*-Fluoren-9-yl)methyl *S*-3-(4-*tert*-butoxyphenyl)-1-oxo-1-((*R*)-1-phenylethylamino)propan-2-ylcarbamate (3b). White microcrystals (74%); mp 180.1-181.1; $[\alpha]^{24}_D$ = +8.6° (*c* = 1.0, $CHCl_3$); ^{1}H NMR δ 7.81 (d, *J* = 7.5 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.45 (t, *J* = 6.9 Hz, 2H), 7.38-7.26 (m, 5H), 7.21-7.15 (m, 4H), 6.98 (d, *J* = 7.8 Hz, 2H), 5.76 (d, *J* = 5.1 hz, 1H), 5.53 (d, *J* = 5.5 Hz, 1H), 5.04 (quintet, *J* = 6.9 Hz, 1H), 4.45-4.41 (m, 3H), 4.24 (t, *J* = 6.6 Hz, 1H), 3.18 (br s, 1H), 2.99-2.96 (m, 1H), 1.42-1.31 (m, 12H); ^{13}C NMR 169.6, 154.5, 143.8, 143.7, 142.8, 141.3, 129.9, 128.7, 127.8, 127.5, 127.1, 126.0, 125.1, 124.4, 120.0, 78.5, 77.3, 67.0, 56.6, 49.0, 47.1, 38.5, 28.8, 21.6. Anal. Calcd for $C_{36}H_{40}N_2O_5 \cdot H_2O$: C, 74.46; H, 6.94; N, 4.82; Found: C, 74.49; H, 7.07; N, 4.58.

(9*H*-Fluoren-9-yl)methyl *S*-3-methyl-1-oxo-1-((*S*)-1-phenylethylamino)butan-2-ylcarbamate (4a). White powder (77%); mp 166.3-168.8 °C; $[\alpha]^{25}_D$ = +22.1° (*c* = 2.2, $CHCl_3$); ^{1}H NMR δ 7.75 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.2 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.34-7.27 (m, 7H), 6.24 (d, *J* = 7.8 Hz, 1H), 5.44 (d, *J* = 8.7 Hz, 1H), 5.18-5.06 (m, 1H), 4.43-4.23 (m, 2H), 4.22-4.13 (m, 1H), 4.00-3.90 (m, 1H), 2.21-2.06 (m, 1H), 1.48 (d, *J* = 6.6 Hz, 3H), 0.97 (two overlapped doublets, *J* = 7.7 Hz, 6H); ^{13}C NMR 170.1, 169.9, 143.8, 142.6, 141.3, 128.7, 127.7, 127.4, 127.1, 126.1, 125.0, 119.9, 67.0, 60.5, 48.9, 47.1, 31.1, 21.7, 19.2, 18.0. Anal. Calcd for $C_{28}H_{30}N_2O_3$: C, 75.99; H, 6.83; N, 6.33; Found: C, 76.10; H, 7.01; N, 6.51.

(9*H*-Fluoren-9-yl)methyl *S*-3-(4-*tert*-butoxyphenyl)-1-oxo-1-((*S*)-1-phenylethylamino)propan-2-ylcarbamate (4b). White powder (79%); mp 196.3-197.7 °C; $[\alpha]^{24}_D$ = -6.2° (*c* = 1.0, $CHCl_3$); ^{1}H NMR δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.56 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.33-7.22 (m, 5H) 7.12 (d, *J* = 7.2 Hz, 2H), 7.00-6.97 (m, 2H), 6.83 (d, *J* = 8.1 Hz, 2H), 5.88 (br s, 1H), 5.42 (br s, 1H), 5.06-5.01 (m, 1H), 4.45-4.35 (m, 2H), 4.23-4.13 (m, 1H), 3.11-3.00 (m, 1H), 2.98-2.86 (m, 1H), 1.91-1.82 (m, 1H), 1.39 (d, *J* = 6.9 Hz, 3H), 1.31 (s, 9H); ^{13}C NMR 169.9, 154.6, 143.9, 142.6, 141.5, 130.0, 128.8, 127.9, 127.6, 127.3, 126.3, 125.2, 124.5, 120.2, 78.6, 77.4, 67.2, 56.6, 49.2, 47.3, 38.3, 29.0, 21.7. Anal. Calcd for $2(C_{72}H_{78}N_4O_9) \bullet H_2O$: C, 75.63; H, 6.88; N, 4.90; Found: C, 75.59; H, 6.82; N, 4.77.

(9*H*-Fluoren-9-yl)methyl *S*-3-methyl-1-oxo-1-(1-phenylethylamino)butan-2-ylcarbamate (3a+4a). Yellow oil (74%); ^{1}H NMR δ 7.69 (d, *J* = 7.5 Hz, 2H), 7.52 (d, *J* = 7.2 Hz, 2H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.34-7.12 (m, 21H), 6.01 (s, 0.46H), 5.15-5.05 (m, 1.58H), 3.94 (t, *J* = 6.5 Hz, 1H), 3.74 (q, *J* = 6.0 Hz, 1H), 3.16 (t, *J* = 3.0 Hz, 2H), 2.96 (dd, *J* = 12.0, 6.2 Hz, 1H), 2.82 (dd, *J* = 12.2, 6.9 Hz, 1H), 2.34-2.16 (m, 2H), 1.42 (d, *J* = 6.9 Hz, 6H), 1.24 (d, *J* = 6.6 Hz, 3H), 0.91 (t, *J* = 7.5 Hz, 6H), 0.78 (d, *J* = 6.9 Hz, 6H), 0.68 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR 173.8, 173.7, 146.4, 146.4, 146.0, 144.0, 143.8, 141.6, 141.6, 129.1, 129.0, 129.0, 128.8, 127.6, 127.6, 127.5, 127.4, 127.2, 127.1, 126.6, 126.5, 125.0, 125.0, 121.4, 120.3, 120.2, 120.1, 60.4, 60.4, 58.6, 51.1, 48.5, 48.4. HRMS calcd for $C_{28}H_{30}N_2O_3$ $[M+H-CO_2]^+$ 399.2436, found 399.2393

(9*H*-Fluoren-9-yl)methyl *S*-3-(4-*tert*-butoxyphenyl)-1-oxo-1-(1-phenylethylamino)propan-2-ylcarbamate (3b+4b). Off-white powder (66%); mp 170.8-176.8 °C; ^{1}H NMR δ 7.76 (d, *J* = 6.9 Hz, 4H), 7.55 (d, *J* = 6.9 Hz, 4H), 7.40 (t, *J* = 7.0 Hz, 4H), 7.34-7.20 (m, 12H), 7.18-7.06 (m,

6H), 6.92 (d, *J* = 7.8 Hz, 2H), 6.83 (d, *J* = 8.1, 2H), 5.94 (br s, 1H), 5.74 (br s ,1H), 5.50 (br s, 2H), 5.30-4.92 (m, 2H), 4.46-4.26 (m, 6H), 4.22-4.12 (m, 2H), 3.22-2.78 (m, 4H), 1.33 (s, 18H), 1.32 (overlapped d, J = 6.3 Hz, 6H); ^{13}C NMR 169.8, 169.6, 154.4, 154.4, 143.7, 143.7, 142.4, 142.4, 141.3, 129.9, 129.8, 128.6, 128.6, 127.7, 127.4, 127.4, 127.1, 126.1, 126.0, 125.0, 124.4, 124.3, 120.0, 78.5, 78.4, 77.2, 67.0, 56.6, 48.9, 47.1, 38.5, 38.1, 28.8, 21.5. HRMS calcd for $C_{36}H_{38}N_2O_4$ $[M+H]^+$ 563.2910, found 563.2904.

Acknowledgements

We thank Dr. C. Dennis Hall for his useful suggestions.

References

1. Han, S.-Y.; Kim Y.-A. *Tetrahedron* **2004**, *60*, 2447.
2. (a) Katritzky, A. R.; He, H.-Y.; Suzuki, K. *J. Org. Chem.* **2000**, *65*, 8210. (b) Katritzky, A. R.; Tala, S. R.; Singh, S. K. *Synthesis* **2006**, *19*, 3231. (c) Katritzky, A. R.; Chen, Q.-Y.; Tala, S. R. *Org. Biomol. Chem.* **2008**, In press (DOI: 10.1039/b802846j). (d) Katritzky, A. R.; Hoffmann, S.; Suzuki, K. *ARKIVOC* **2004**, (*xii*), 14.
3. Katritzky, A. R.; Shestopalov, A. A.; Suzuki, K. *Synthesis* **2004**, *11*, 1806.
4. (a) Katritzky, A. R.; Le, K. N. B.; Khelashvili, L.; Mohapatra, P. P. *J. Org. Chem.* **2006**, *71*, 9861. (b) Katritzky, A. R.; Pastor, A. *J. Org. Chem.* **2000**, *65*, 3679. (c) Katritzky, A. R.; Abdel-Fattah, A. A. A.; Wang, M. *J. Org. Chem*. **2003**, *68*, 1443. (d) Katritzky, A. R.; Fang, Y.; Donkor, A.; Xu, J. *Synthesis* **2000**, *14*, 2029. (e) Katritzky, A. R.; Suzuki, K.; Singh, S. K.; He, H.-Y. *J. Org. Chem*. **2003**, *68*, 5720. (f) Katritzky, A. R.; Suzuki, K.; Singh, S. K. *Croat. Chem. Acta* **2004**, *77*, 175.
5. (a) Katritzky, A. R.; Angrish, P.; Hür, D.; Suzuki, K. *Synthesis* **2005**, *3*, 397. (b) Katritzky, A. R.; Wang, M.; Yang, H.; Zhang, S.; Akhmedov, N. G. *ARKIVOC* **2002**, (*viii*), 134. (c) Katritzky, A. R.; Khashab, N. M.; Yoshioka, M.; Haase, D. N.; Wilson, K. R.; Johnson, J. V.; Chung, A.; Haskell-Luevano, C. *Chem. Biol. and Drug Des.* **2007**, *70*, 465. (d) Katritzky, A. R.; Angrish, P. *Synthesis* **2006**, *24*, 4135. (e) Katritzky, A. R.; Angrish, P.; Suzuki, K. *Synthesis* **2006**, *3*, 411.
6. Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc*. **1964**, *86*, 1839.
7. Gagnon, P.; Huang, X.; Therrien, E.; Keillor, J. W. *Tetrahedron Lett*. **2002**, *43*, 7717.
8. Katritzky, A. R.; Todadze, E.; Angrish, P.; Draghici, B. *J. Org. Chem.* **2007**, *72*, 5794.
9. Katritzky, A. R.; Narindoshvili, T.; Draghici, B.; Angrish, P. *J. Org. Chem.* **2008**, *73*, 511.