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Preparation of C_{60}-based active esters and coupling of C_{60} moiety to amines or alcohols

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Abstract—We report the syntheses of C_{60}-based active esters and the coupling of their C_{60} moiety to various amines or alcohols. Methano[60]fullerene carboxylic acid was activated by esterification with *N*-hydroxysuccinimide (NHS) or pentafluorophenol (PFP) and the active esters were isolated. Reactions of the active esters with amines or alcohols proceeded easily to give a variety of compounds having the C_{60} moiety.
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[60]Fullerene derivatives have a wide variety of biological activities such as neuroprotective, enzyme inhibition, photoinduced DNA cleavage, and antigenicity.[1] To apply functionalized fullerenes in material science and medicinal chemistry, many fullerene derivatives have been synthesized by using reactions that involve addition to C_{60}.[2] Methano[60]fullerenes are one of the most well-studied fullerene derivatives, and several methods are available for their synthesis: (i) the Bingel reaction, (ii) the addition–elimination reactions of sulfonium ylides or phosphonium ylides, and (iii) the addition-thermal decomposition of diazo compounds.[3] However, these methods have some limitations in terms of molecular design, namely, method (i) requires strong basic condition, and the preparation of ylides and diazo compounds is difficult in methods (ii) and (iii), respectively. These methods also do not give a pure mono-adduct in high yield due to the formation of isomers and multi-adducts. On the other hand, a series of methano[60]fullerene carboxylic acids are useful to obtain various esters and amides by condensation reactions with alcohols and amines, respectively, using a condensing reagent. Further, the in situ formation of acid chloride from methano[60]fullerene carboxylic acid and the subsequent reaction with alcohol and amine are also useful.[1d,3e,4] However, the carboxylic acid derivatives are insoluble in most organic solvents. Two approaches have been reported to improve solubility. One is the synthesis and isolation of the acid chloride from methano[60]fullerene carboxylic acid, which in turn is converted into amide or ester by reacting with amine or alcohol, respectively.[5] The other is the use of succinimide ester that can react with molecules containing primary and secondary amino groups.[6] The acid chloride and the succinimide ester are both isolable and soluble compounds. Although these methods are convenient, it is difficult to induce the selective reaction of the acid chloride with alcohol or amine due to its high reactivity, forming both ester and amide. Further, the condensation reaction of succinimide ester with alcohol has not been reported.

In this paper, we report a simple and efficient method for the preparation of C_{60} derivatives coupled with amines and alcohols. Methano[60]fullerene carboxylic acid was chosen and its carboxylic acid group was activated to facilitate the formation of the corresponding amide or ester. Condensation reactions using isolated succinimide ester containing C_{60} moiety **1a** have been reported.[6] According to that paper, succinimide ester **1a** was synthesized from fulleromalonic acid $C_{61}(COOH)_2$ and *N*-hydroxysuccinimide (NHS) by the DCCI method with decarboxylation in only 30% yield.[6a] We synthesized **1a** via the condensation reaction of monoacid **1** with NHS using EDCI instead of DCCI, and achieved an improved yield (56%) (Scheme 1). Monoacid **1** was

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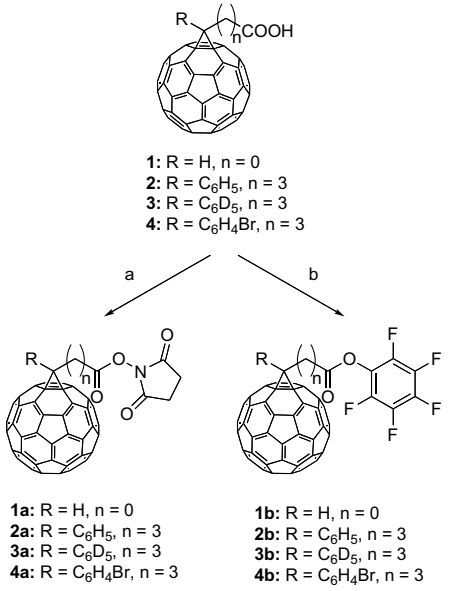
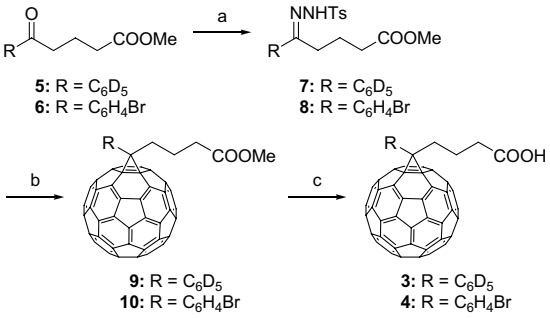
prepared from *tert*-butyl [60]fullerene acetate according to the procedure reported in the literature.[3b,e,7] To improve the coupling efficiency of the active ester with amines and alcohols, pentafluorophenol (PFP) ester **1b** was prepared in 71% yield (Scheme 1). PFP ester is highly reactive and is commonly used for the derivatization of primary and secondary amino groups.[8] Furthermore, to obtain various types of intermediates, another type of methano[60]fullerene carboxylic acid **2** was chosen and activated to give active esters **2a** and **2b** in 72% and 91% yields, respectively (Scheme 1). Acid **2** was synthesized from 4-benzoylbutyric acid in four steps according to a procedure reported in the literature.[5a,9] The phenyl ring in **2** has the advantage of further modification, such as introduction of various substituents and replacement of hydrogen with deuterium. Acid **3**, whose phenyl ring hydrogens were converted into deuteriums, was also synthesized from methyl 4-benzoylbutyrate-d_5 **5** in three steps with the procedure used for **2** (Scheme 2). Methyl ester **5** was purchased from Spectra Stable Isotopes, Inc. Acid **4** containing bromo atom was synthesized from methyl 4-(4-bromobenzoyl)butyrate **6** in three steps. Acids **3** and **4** were activated by esterification with NHS or PFP using EDCI in carbon disulfide (CS_2), and active esters **3a**, **4a**, **3b**, and **4b** were isolated in 53%, 67%, 43%, and 91% yields, respectively. These active esters containing the C_{60} moiety were used as the key intermediate to produce a variety of methano[60]fullerene derivatives efficiently.

The derivatization reactions of the isolated active esters toward various amines were demonstrated. First, the active esters were allowed to react with benzylamine to give the corresponding amides **11** (Table 1, entries 1–8). Both NHS ester **1a** and PFP ester **1b** smoothly reacted with benzylamine in quantitative yield (Table 1, entries 1 and 5). In contrast, NHS ester



Scheme 1. Syntheses of a series of active esters containing C_{60} moiety from acids **1**–**4**. Reagents, conditions, and yields: (a) NHS, EDCI, dry THF or CS_2, rt, 53–72%; (b) PFP, EDCI, dry THF or CS_2, rt, 43–91%.



Scheme 2. Syntheses of methano[60]fullerene carboxylic acids **3** and **4**. Reagents, conditions, and yields: (a) $TsNHNH_2$, MeOH, reflux, 81–88%; (b) C_{60}, NaOMe, pyridine, 1,2-dichlorobenzene, reflux, 39–47%; (c) HCl/AcOH, toluene, reflux, 84–94%.

Table 1. Derivatization with amines

Entry	Amine	Active ester	Equiv[a]	Time (h)	Yield (%)
1		**1a**	1.2	2	100
2		**2a**	1.6	2	75
3		**2a**	0.1	1	100[b]
4	H_2N	**4a**	1.0	9	97
5		**1b**	1.4	1	92
6		**2b**	1.6	25	48
7		**2b**	0.1	1	54[b]
8		**4b**	1.0	17	73
9		**1a**	0.1	19	81[b]
10		**2a**	0.1	42	89[b]
11	H_2N COOEt	**1b**	0.1	22	76[b]

[a] Equivalent amount of active ester to amine.
[b] The isolated yield is based on the amount of active ester.

2a reacted in moderate yield (Table 1, entry 2); however, the yield was improved by the reaction with 10 equiv of amine (Table 1, entry 3). Although PFP esters were synthesized to achieve high reactivity to amines, PFP esters **2b** and **4b** were less reactive than NHS esters **2a** and **4a** (entries 2–4 and 6–8). These results are not consistent with our expectation that PFP ester is more reactive than NHS ester. Next, the derivatization with amino acid derivative (10 equiv) was carried out to give the corresponding amides in moderate yields (Table 1, entries 9–11).

To investigate the reactivity of the active esters to alcohol, the derivatization reactions with benzyl alcohol (10 equiv) were carried out using NHS ester **1a** or PFP ester **1b**. Under the same conditions as those used for benzylamine, corresponding ester **12** was not obtained. However, on adding DMAP (1 equiv) to the reaction mixture, the esterification was facilitated to give corresponding ester **12** in 72% and 100% yields, respectively (Fig. 1). Even when a mixture of benzylamine (10 equiv) and benzyl alcohol (10 equiv) was allowed to react with NHS ester **1a** or PFP ester **1b**, only corresponding amide **11** was obtained in 100% and 85% yields, respectively. On the other hand, in the presence of DMAP, a mixture of amide **11** and ester **12** was obtained at the ratio of

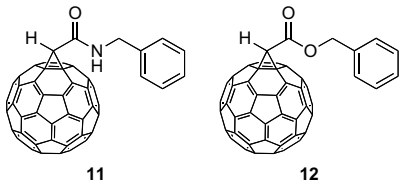


Figure 1. Structures of compounds **11** and **12**.

12:1. These results demonstrate that the active esters selectively react with amine in the absence of DMAP.

Among the different classes of fullerene derivatives, fullerenes coupled with amino acids or peptides are interesting molecules for biological applications.[4a,6b,10] In this regard, there is a need to develop efficient methods for the preparation of fullerenes coupled with amino acids or peptides to widen the applications of fullerenes. We demonstrate here the derivatization of fullerenes with various amino acids and peptides, which involves the following steps. A solution of the NHS ester in DMF was added to an aqueous solution of amino acids or peptides. The reaction mixture was stirred with vortex mixer for 10 s, left at room temperature for 1–6 h, and subjected to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) analysis without further purification. The MALDI-TOF-MS were acquired in the negative ion mode using α-cyano-4-hydroxycinnamic acid (CHCA) as the MALDI matrix. The results of the MALDI-TOF-MS analysis summarized in Table 2 indicate that the NHS esters reacted with the free amino functionality of amino acids and peptides under mild conditions. A typical MALDI-TOF-MS is shown in Figure 2(a). Leucine-enkephalin (YGGFL) was derivatized with NHS ester **1a**. M^- ion of the derivatized peptide was observed at *m*/*z* 1315.16 (calcd mass: 1315.26). Signals which appeared at *m*/*z* 600–800 were those derived from the matrix and the reagent. Figure 2(b) shows the reverse-phase HPLC chromatogram of the reaction mixture at 6 h after reaction, which was obtained with a C_4 RP column. The native peptide (4.1 min) was not detected while the derivatized peptide with **1a** was detected at 24.1 min. The broad peak at 26–31 min was derived from the unreacted reagent **1a**. The results of MALDI-TOF-MS analysis and RP-HPLC analysis indicated that NHS ester **1a** reacted with pentapeptide quantitatively.

Table 2. Derivatization with amino acids and peptides

Entry	Amino acid and peptide	Active ester	Monoisotopic MW of derivative	
			Expected	Observed
1	Gly	**1a**	835.03	835.02
		2a	953.11	953.12
		3a	958.14	958.11
		4a	1031.02	1031.03
2	Gly-Gly-Gly	**1a**	949.07	948.94
		2a	1067.15	1067.00
3	Z-Lys	**1a**	1040.14	1040.13
		2a	1158.22	1158.15
4	Bz-Gly-Lys	**1a**	1067.15	1067.16
		2a	1185.23	1185.19
5	Gly-Gly-Tyr-Arg	**1a**	1211.21	1211.04
		2a	1329.29	1329.04
6	Tyr-Gly-Gly-Phe-Leu	**1a**	1315.26	1315.16
		2a	1433.34	1433.10

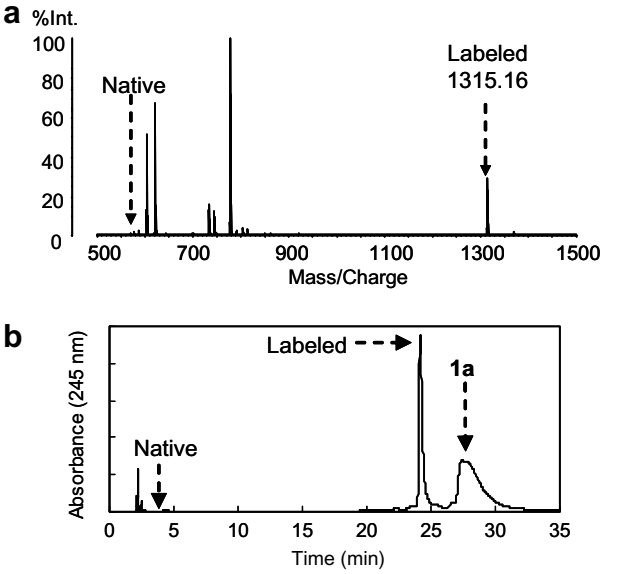


Figure 2. (a) MALDI-TOF-MS of leucine-enkephalin (YGGFL) derivatized with **1a**. M^- ion (calculated mass 1315.26) of the derivatized peptide was observed at *m*/*z* 1315.16. The spectrum was acquired in the reflectron negative mode using CHCA as the MALDI matrix. (b) RP-HPLC chromatogram of the reaction mixture of YGGFL derivatized with active ester **1a** at 6 h. Inertsil WP300 C_4 column from GL Sciences was used.

Spectral data of new compounds were described in Ref. 11. General procedure for the derivatization with amines was described in Ref. 12.

In summary, NHS esters and PFP esters of methano[60]fullerene carboxylic acids were synthesized and their C_{60} moiety was coupled to molecules containing amino or hydroxyl groups. The active ester reacted with amine selectively and only reacted with alcohol in the presence of DMAP. MALDI-TOF-MS analysis indicated the potential utility of these active esters for the preparation of fullerene-modified amino acids or peptides for application in biological studies. This approach features ease of coupling of the C_{60} moiety to a variety of molecules with amino or hydroxyl groups via a simple reaction under mild conditions. These [60]fullerene-based active esters have the potential to be used as building blocks for the synthesis of various methano[60]fullerene derivatives, extending the application of fullerene in medicinal chemistry and material science. Based on these ideas, expecting the biological activities, we have examined the preparation of some amino acids and related derivatives having C_{60} moiety. Results will be appeared elsewhere soon. Also the applications of our reagents **1a**–**4a** and **1b**–**4b** for mass spectrometry of small amine molecules are in progress.

Acknowledgment

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References and notes

1. (a) Dugan, L. L.; Turetsky, D. M.; Du, C.; Lobner, D.; Wheeler, M.; Almli, C. R.; Shen, C. K.-F.; Luh, T.-Y.; Choi, D. W.; Lin, T.-S. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 9434; (b) Friedman, S. H.; De Camp, D. L.; Sijbesma, P. R.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506; (c) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918; (d) Chen, B.-X.; Wilson, S. R.; Das, M.; Coughlin, D. J.; Erlanger, B. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 10809.
2. (a) Maggini, M.; Scorrano, G.; Prato, M. *J. Am. Chem. Soc.* **1993**, *115*, 9798; (b) Prato, M.; Maggini, M.; Giacometti, C.; Scorrano, G.; Sandona, G.; Farnia, G. *Tetrahedron* **1996**, *52*, 5221; (c) Yamakoshi, Y.; Yagami, T.; Sueyoshi, S.; Miyata, N. *J. Org. Chem.* **1996**, *61*, 7236; (d) Nakamura, E.; Yamago, S. *Acc. Chem. Res.* **2002**, *35*, 867.
3. (a) Bingel, C. *Chem. Ber.* **1993**, *126*, 1957; (b) Wang, Y.; Cao, J.; Schuster, D. I.; Wilson, S. R. *Tetrahedron Lett.* **1995**, *36*, 6843; (c) Bestmann, H. J.; Hadawi, D.; Roder, T.; Moll, C. *Tetrahedron Lett.* **1994**, *35*, 9017; (d) Isaacs, L.; Wehrsig, A.; Diederich, F. *Helv. Chim. Acta.* **1993**, *76*, 1231; (e) Isaacs, L.; Diederich, F. *Helv. Chim. Acta.* **1993**, *76*, 2454.
4. (a) Prato, M.; Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Wudl, F. *J. Org. Chem.* **1993**, *58*, 5578; (b) Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palú, G.; Blair, E. D. *J. Med. Chem.* **1994**, *37*, 4558; (c) Giacalone, F.; Segura, J. L.; Martin, N. *J. Org. Chem.* **2002**, *67*, 3529.
5. (a) Hummelen, J. C.; Knight, B. W.; LePeg, F.; Wudl, F.; Yao, J.; Wilkins, C. L. *J. Org. Chem.* **1995**, *60*, 532; (b) Ito, H.; Tada, T.; Sudo, M.; Ishida, Y.; Hino, T.; Saigo, K. *Org. Lett.* **2003**, *5*, 2643.
6. (a) Lamparth, I.; Schick, G.; Hirsch, A. *Liebigs Ann./Recueil* **1997**, *1*, 253; (b) Jung, G.; Redemann, T.; Kroll, K.; Meder, S.; Hirsch, A.; Boheim, G. *J. Peptide Sci.* **2003**, *9*, 784.
7. (a) Sun, Y.-P.; Lawson, G. E.; Riggs, J. E.; Ma, B.; Wang, N.; Moton, D. K. *J. Phys. Chem. A* **1998**, *102*, 5520; (b) Ma, B.; Bunker, C. E.; Guduru, R.; Zhang, X.-F.; Sun, Y.-P. *J. Phys. Chem. A* **1997**, *101*, 5626.
8. Kovacs, J.; Kisfaludy, L.; Ceprini, M. *J. Am. Chem. Soc.* **1967**, *89*, 183.
9. González, R.; Hummelen, J. C.; Wudl, F. *J. Org. Chem.* **1995**, *60*, 2618.
10. (a) Bianco, A.; Da Ros, T.; Prato, M.; Toniolo, C. *J. Pept. Sci.* **2001**, *7*, 208; (b) Pantarotto, D.; Bianco, A.; Pellarini, F.; Tossi, A.; Giangaspero, A.; Zelezetsky, I.; Briand, J.-P.; Prato, M. *J. Am. Chem. Soc.* **2002**, *124*, 12543; (c) Bianco, A.; Pantarotto, D.; Hoebeke, J.; Briand, J.-P.; Prato, M. *Org. Biomol. Chem.* **2003**, *1*, 4141.
11. All the new compounds were characterized on the basis of NMR and mass spectral data. Selected spectroscopic data: **2a**: ^{1}H NMR ($CDCl_3$, 400 MHz, δ; ppm) 7.94 (2H, m), 7.58–7.49 (3H, m), 3.00 (2H, m), 2.85–2.81 (6H, m), 2.31 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 993.10, found M^-: 993.01.
3a: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 2.99 (2H, m), 2.85–2.81 (6H, m), 2.31 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 998.13, found M^-: 998.11.
4a: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.75 (2H, d, $J = 8.5$ Hz), 7.61 (2H, d, $J = 8.5$ Hz), 2.90 (2H, t, $J = 8.2$ Hz), 2.78–2.75 (6H, m), 2.25–2.20 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 1071.01 and $[M+2]^-$: 1073.01, found M^-: 1071.01 and $[M+2]^-$: 1072.99.
1b: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 5.09 (1H, s). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 943.99, found M^-: 944.04.
2b: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.95 (2H, d, $J = 7.0$ Hz), 7.57 (2H, t, $J = 7.3$ Hz), 7.49 (1H, t, $J = 7.3$ Hz), 3.00 (2H, m), 2.88 (2H, t, $J = 7.3$ Hz), 2.33 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative) calculated molecular ion M^-: 1062.07, found M^-: 1062.01.
3b: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 3.00 (2H, m), 2.88 (2H, m), 2.33 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 1067.10, found M^-: 1067.07.
4b: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.76 (2H, d, $J = 8.5$ Hz), 7.63 (2H, d, $J = 8.5$ Hz), 2.91 (2H, t, $J = 8.2$ Hz), 2.82 (2H, t, $J = 7.3$ Hz), 2.26–2.22 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 1139.98 and $[M+2]^-$: 1141.98, found M^-: 1139.22 and $[M+2]^-$: 1141.92.
3: MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 901.12, found M^-: 901.08.
4: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.78 (2H, d, $J = 8.5$ Hz), 7.64 (2H, d, $J = 8.5$ Hz), 2.88 (2H, t, $J = 8.2$ Hz), 2.54 (2H, t, $J = 7.3$ Hz), 2.20–2.13 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 973.99 and $[M+2]^-$: 975.99, found M^-: 973.99 and $[M+2]^-$: 975.97. **7**: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.20 (1H, s), 7.92 (2H, d, $J = 8.2$ Hz), 7.30 (2H, d, $J = 8.2$ Hz), 3.81 (3H, s), 2.63 (2H, m), 2.41 (3H, s), 2.33 (2H, m), 1.69 (2H, m). MS (EI) *m/z* 379 (M). Mp: 125–126 °C.
8: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 9.24 (1H, s), 7.90 (2H, d, $J = 8.5$ Hz), 7.52 (2H, d, $J = 8.5$ Hz), 7.46 (2H, d, $J = 8.5$ Hz), 7.30 (2H, d, $J = 8.5$ Hz), 3.81 (3H, s), 2.59 (2H, t, $J = 8.2$ Hz), 2.41 (3H, s), 2.33 (2H, t, $J = 6.1$ Hz), 1.69–1.64 (2H, m). MS (EI): *m/z* 452 (M), 454 (M + 2). Anal. Calcd for $C_{19}H_{21}BrN_2O_4S$: C, 50.34; H, 4.67; N, 6.18. Found: C, 50.36; H, 4.71; N, 6.08. Mp: 151–155 °C.
9: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 3.68 (3H, s), 2.91 (2H, m), 2.53 (2H, t, $J = 7.6$ Hz), 2.19 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 915.13, found M^-: 915.14.
10: ^{1}H NMR ($CDCl_3$, 500 MHz, δ; ppm) 7.82 (2H, d, $J = 8.5$ Hz), 7.68 (2H, d, $J = 8.5$ Hz), 3.69 (3H, s), 2.89 (2H, t, $J = 8.5$ Hz), 2.54 (2H, t, $J = 7.6$ Hz), 2.20–2.15 (2H, m). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 988.01 and $[M+2]^-$: 990.01, found M^-: 987.91 and $[M+2]^-$: 989.87.
12. General procedure for the derivatization with amine. To a solution of **2a** (15 mg, 0.015 mmol) in $CHCl_3$ (4 mL) was added 0.18 M solution of benzylamine in $CHCl_3$ (0.85 mL, 0.15 mmol). The reaction mixture was stirred at room temperature for 1 h and then subjected to flash column chromatography on silica gel using a toluene/$CHCl_3$ mixture (1:1) as eluent to give the corresponding amide as a brown solid (16.6 mg, quant). MALDI-TOF-MS (matrix: CHCA, reflectron negative): calculated molecular ion M^-: 985.15, found M^-: 985.10.