A Facile Two-Step Synthesis of Novel Ring-A Double Substituted Tryptophan Building Blocks for Combinatorial Chemistry

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Abstract: A fast synthesis of ring-A disubstituted Fmoc and Boc protected L-tryptophan analogs was achieved starting from the appropriate 2,4- or 2,3-disubstituted phenylhydrazines and optically active N,N-diprotected L-glutamic acid γ-aldehydes, utilizing a Fischer-indole synthesis as a key step. Unlike most of the previously reported methods, that required the multistep stereoselective generation of a chiral carbon, this fast methodology is useful for generating optically active ring-A disubstituted protected tryptophans starting from a simple and common chiral precursor. These building blocks have a wide application scope in peptide and combinatorial chemistry fields.

Key words: Boc strategy, combinatorial chemistry, Fischer indole synthesis, Fmoc strategy, orthogonal protection, tryptophan

Combinatorial chemistry is based on the simple assumption that the greater the diversity of compounds tested, the better the chance of finding one that can be developed into a drug or other industrial lead. The scope of this approach is limited to a finite number of molecules as a result of the limited organic reactions available for synthesis on solid supports and by the limited availability of multi-reagent organic reactions. A main goal of the present work is to develop a new tryptophan-based building block for combinatorial chemistry. These compounds can be used as building blocks for modifications of peptides,[1,2] for preparation of new types of natural indole[3,4] and bis-indole[5,6] alkaloids, for the synthesis of indoleamine 2,3-dioxygenase (IDO)[7] or somatostatin[8] inhibitors.

In previous work, ring-A double substituted tryptophans have been reported.[9,10] Optically active ring-A mono-substituted tryptophan analogs were obtained after synthesis and resolution of racemic compounds or by tedious multistep stereoselective alkylation of Schollkopf's auxiliary with moderate stereoselectivity. Thus, a regiospecific bromination of N-protected 3-methyl indoles with NBS under free radical conditions (AIBN) led to the precursor 3-bromomethyl indoles[9,11–13] whose reaction with the anion pre-formed from a Schöllkopf's auxiliary[14] provided enantiomerically enriched products. The desired *trans* diastereomer was subsequently hydrolyzed under acidic conditions to afford the ring-A mono-substituted tryprophans. In a similar approach the optically active tryptophan analogs were obtained via a palladium-catalyzed heteroannulation reaction of substituted *o*-iodoanilines with a preformed Schollkopf's auxiliary.[10] An internal alkyne was prepared via alkylation of the Schöllkopf's auxiliary with diphenyl (triethylsilyl)propargyl phosphate. After the heteroannulation a mixture containing L-isotryptophan as minor product was obtained. The desired L-tryptophan was isolated after separation from the minor isomer by column chromatography. In a third approach 5-hydroxy derivatives of tryptophan were prepared via a Fischer indole ring formation mediated by isolated intermediate hydrazones obtained from the condensation of substituted phenylhydrazines and aldehyde derivatives of protected glutamic acid.[15–17]

Overall, these methods suffer from a number of disadvantages. Some are limited to tryptophan analogs with no alkyl substituents on the ring A,[9,11–13] as the presence of such alkyl substitutents will likely promote a non-selective bromination and a mixture of di-halogenated indole products will be obtained. In general, these methods are tedious multistep syntheses, involving the problematic separation of isomers, which are obtained in the alkylation and annulation reactions respectively, underlining the main drawbacks of these approaches. Finally, the Fischer-indole approach, which requires the isolation of the intermediate hydrazone, was limited to a single hydroxyl substituent on the ring-A and led to the formation of *N*-acetyl or *N*-phtalimido tryptophans, which are not appropriately protected for peptide or combinatorial chemistry. Nevertheless, this approach seems to be the most versatile and rapid of the available strategies.

The limitations of the current approaches and the lack of a specific general procedure for accessing di-substituted A-ring tryptophans, prompted us to develop an improved method for the preparation of these building blocks, which would generalize the synthesis, preferably reduce the number of synthetic steps and produce properly protected building blocks, that are applicable to SPPS, SPOC and parallel synthesis. Our approach for the synthesis of ring-A disubstituted tryptophans is based on a modification and extension of the simple Fischer-indole reaction between 2,4- or 2,3-disubstituted phenylhydrazine hydrochlorides and optically active *N*,*N*-diprotected glutamic acid γ-aldehydes **4**, **12** and **16**. Therefore, aldehydes properly protected for Boc and Fmoc strategies, were required to achieve our synthetic goal.

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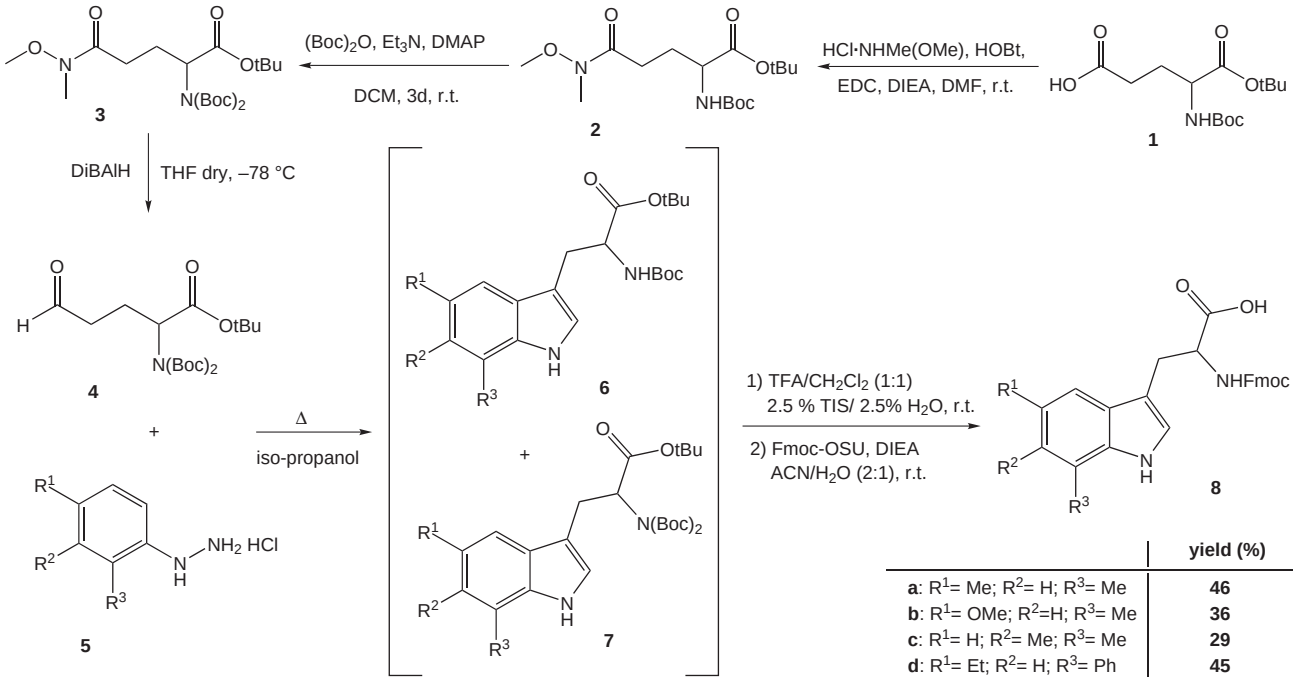


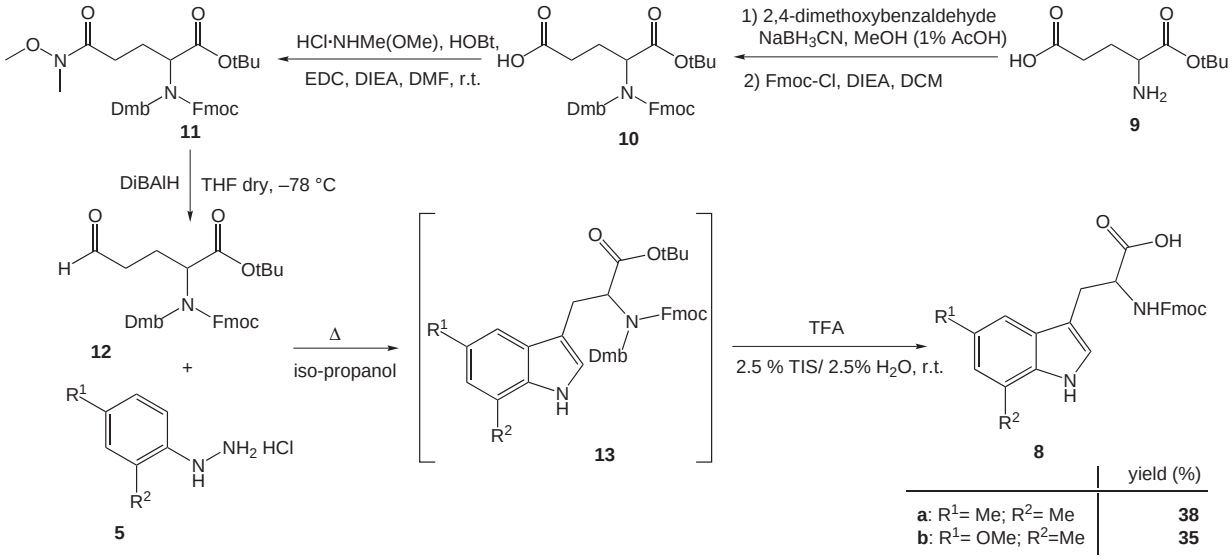
Scheme 1

Evidently, the double protection of the α-amino group in the γ-aldehyde precursors was necessary in order to avoid the undesired intramolecular lactamization of the obtained γ-aldehydes,[18] preventing racemization of the asymmetric carbon. In the light of this need, we have synthesized various aldehydes carrying orthogonal protecting groups on the α-amino and α-carboxylic moieties starting from the L-glutamic acid counterpart (**4**, **12** and **16**).

The synthesis of N,N-di-Boc protected aldehydes **4** and **16** is known.[19,20] Our attempts to follow literature procedures resulted in low yields, therefore aldehydes **4** and **16** were synthesized by combination of different strategies. Thus, γ-aldehyde **4** (see Scheme 1) was prepared starting from N-Boc protected L-glutamic acid *t*-butyl ester **1**, which was converted into hydroxamate **2** (Weinreb amide) by reaction with *N*,*O*-dimethylhydroxylamine hydrochloride in the presence of EDC/DIEA. The second protection with $(Boc)_2O$ was performed in dichloromethane in the presence of triethylamine and a catalytic amount of 4-dimethylaminopyridine.[21] Reduction with DiBAlH at –78 °C in dry THF led to the formation of γ-aldehyde **4** (final yield after three steps 55%). L-Glutamic acid dimethyl ester **14** was simultaneously double protected with $(Boc)_2O$ in dichloromethane in the presence of triethylamine and a catalytic amount of 4-dimethylaminopyridine (see Scheme 3). Reduction with DiBAlH led to the formation of γ-aldehyde **16** (final yield 39%). γ-Aldehyde **12** was prepared from L-glutamic acid *t*-butyl ester **9** (see Scheme 2). The first protection of **9** was performed via reductive alkylation of α-amino group with acid labile 2,4-dimethoxybenzaldehyde in the presence of $NaCNBH_3$ in methanol containing 1% of acetic acid.[22] The second protection to obtain **10** was carried out without purification in dichloromethane using Fmoc-Cl in the presence of DIEA (yield after two steps: 65%). The intermediate *N*,*N*-diprotected glutamic acid α-*t*-butyl ester **10** was converted into hydroxamate **11**, which was further reduced with DiBAlH to afford aldehyde **12**.

The formation of disubstituted tryptophan esters by Fischer indole reaction was achieved by reflux in *iso*-propanol,[23] simplifying significantly the production of the building blocks (previously obtained only after isolation of intermediate hydrazones). Thus, 2,3- and 2,4-disubstituted phenylhydrazine hydrochlorides[24] (**5**) were refluxed with N,N-di-Boc protected γ-aldehyde **4** in iso-propanol (Scheme 1) to give in one step the expected tryptophan analogs. We noticed that the second unstable N-Boc group was partially cleaved in the presence of 1 equivalent of HCl during the Fischer indole reaction, affording the mixture of mono-Boc/di-Boc tryptophan esters **6** and **7**. This partial deprotection appeared to be non-significant as the mixture was further treated with TFA/CH_2Cl_2 (1:1), leading to the crude unprotected amino acid, which was submitted to further protection using Fmoc-OSu in ACN/H_2O (1:1) in the presence of DIEA leading the desired Fmoc-tryptophans (**8**).[25]

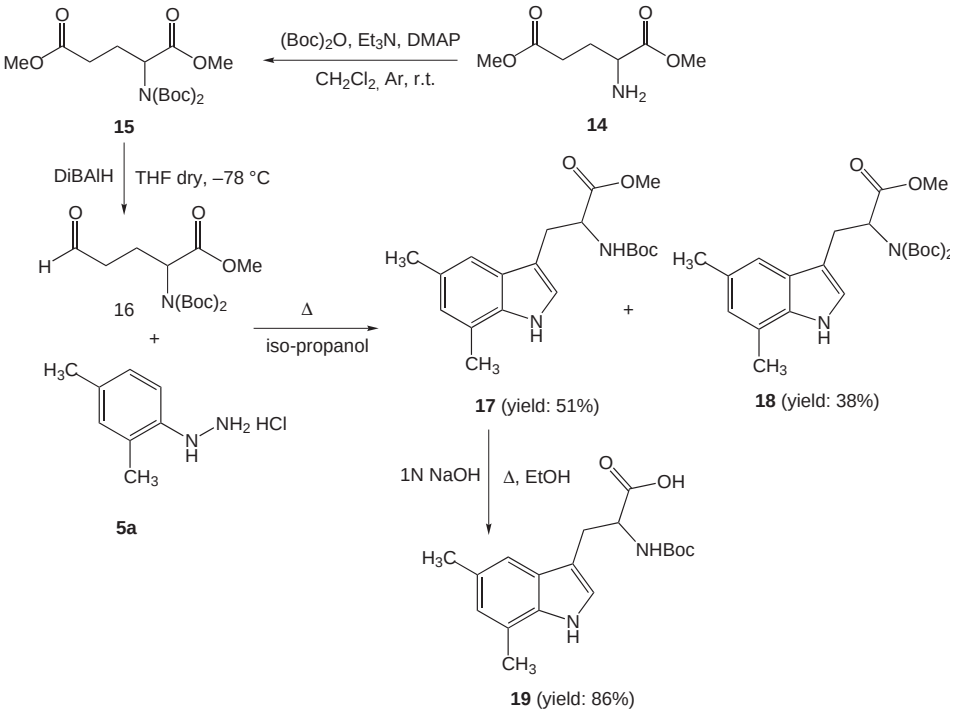
In an alternative approach, the synthesis of Fmoc-protected tryptophan analogs was performed as shown in Scheme 2. Initially prepared γ-aldehyde *t*-butyl ester **12** was refluxed in *iso*-propanol with 2,4-disubstituted phenylhydrazine hydrochlorides (**5a** and **5b**) to give the corresponding protected tryptophan esters (**13a** and **13b**). By this method, the desired disubstituted Fmoc tryptophans (**8a** and **8b**) were obtained through the acidic removal (TFA) of *t*-Bu ester and N-Dmb groups in the final step.



Scheme 2

Based on this successful route to produce disubstituted tryptophans for Fmoc solid-phase chemistry, we decided to develop a general approach for synthesis of the tryptophans for combinatorial synthesis via Boc chemistry on solid support and as well as in solution. For this purpose, γ-aldehyde methyl ester **16** was prepared and refluxed in *iso*-propanol with 2,4-dimethylphenylphenylhydrazine hydrochloride (**5a**), as described (Scheme 3). As expected, the mixture of mono and di-Boc products (**17** and **18**) was obtained again. After separation by flash chromatography, the resultant methyl ester of mono-Boc-5,7-dimethyl tryptophan **17** was hydrolyzed by heating in ethanol with 1 N NaOH to give the corresponding final product **19**.[26] In this approach the formation of undesired di-Boc trypthophan ester reduces the yield of the final product **19**. Attempts to optimize the reaction conditions are in progress. Although, the generation of this mixture during the synthesis could not be prevented, both building blocks are suitable for the same synthetic methods and will bring about the same products in a combinatorial approach after the Boc groups are cleaved.

We have shown a simple and convenient two-step synthesis of disubstituted tryptophan building blocks for combinatorial chemistry. This general methodology allows the quick production of a variety of protected tryptophans and



Scheme 3

simplifies scale up procedure. The key step involves the standard Fischer-indole reaction between the disubstituted phenylhydrazine hydrochlorides and *N*,*N*-diprotected glutamic acid γ-aldehydes that we have improved and simplified for adaption to SPPS and SPOS. The further simple treatment of the protecting groups, affords the formation of ring-A disubstituted α-nitrogen Boc or Fmoc protected tryptophans. These important synthons are useful in peptidomimetics and combinatorial syntheses for the discovery of novel bioactive compounds.

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References

(1) Kobayashi, J.; Ishibashi, M. *Chem. Rev.* **1993**, *93*, 1753.
(2) Braekman, J. C.; Daloze, D.; Moussiaux, B. *J. Nat. Prod.* **1987**, *50*, 99.
(3) Safdy, M. E.; Kurchasova, E.; Schut, R. N.; Vidro, H.; Hong, E. *J. Med. Chem.* **1982**, *25*, 723.
(4) Wright, C. W.; Allen, D.; Cai, Y.; Phillipson, J. D.; Said, I. M.; Kirby, G. C.; Warhurst, D. C. *Phytother. Res.* **1992**, *6*, 121.
(5) Cook, J. M.; LeQuesne, P. W. *Phytochemistry* **1971**, *10*, 437.
(6) Yamazaki, M.; Suzuki, K.; Fijimoto, H.; Akijama, T.; Sankawa, U.; Iitaka, Y. *Chem. Pharm. Bull.* **1980**, *28*, 861.
(7) Botting, N. *Chem. Soc. Rev.* **1995**, *24*, 401.
(8) Berk, S. C.; Rohrer, S. P.; Degrado, S. J.; Birzin, E. T.; Mosley, R. T.; Hutchins, S. M.; Pasternak, A.; Schaeffer, J. M.; Underwood, D. J.; Chapman, K. T. *J. Comb. Chem.* **1999**, *1*, 388.
(9) Liu, R.; Zhang, P.; Gan, T.; Cook, J. M. *J. Org. Chem.* **1997**, *62*, 7447.
(10) Ma, C.; Liu, X.; Li, X.; Flippen-Anderson, J.; Yu, S.; Cook, J. M. *J. Org. Chem.* **2001**, *66*, 4525.
(11) Allen, M. S.; Hamaker, L. K.; La Loggia, A. J.; Cook, J. M. *Synth. Commun.* **1992**, *22*, 2077.
(12) Zhang, P.; Cook, J. M. *Synth. Commun.* **1995**, *25*, 3883.
(13) Zhang, P.; Liu, R.; Cook, J. M. *Tetrahedron Lett.* **1995**, *36*, 9133.
(14) Schöllkopf, U.; Lonsky, R.; Lehr, P. *Liebigs Ann. Chem.* **1985**, 413.
(15) Masumi, F.; Takeuchi, H.; Kondo, S.; Suzuki, K.; Yamada, S. *Chem. Pharm. Bull.* **1982**, *30*, 3831.
(16) Irie, K.; Ishida, A.; Nakamura, T.; Ohishi, T. *Chem. Pharm. Bull.* **1984**, *32*, 2126.
(17) Schirlin, D.; Gerhart, F.; Hornsperger, J. M.; Haman, M.; Wagner, J.; Jung, M. J. *J. Med. Chem.* **1988**, *31*, 30.
(18) Bold, G.; Steiner, H.; Moesch, L.; Walliser, B. *Helv. Chim. Acta* **1990**, *73*, 405.
(19) Kokotos, G.; Padron, J. M.; Martin, T.; Gibbons, W. A.; Martin, V. S. *J. Org. Chem.* **1998**, *63*, 3741.
(20) Constantinou-Kokotou, V.; Magrioti, V.; Markidis, T.; Kokotos, G. *J. Peptide Res.* **2001**, *58*, 325.
(21) 17 g (49.13 mmol) of **2** were dissolved in 300 mL of CH_2Cl_2. 53.60 g (245.65 mmol) of $(Boc)_2O$, 147 mL (1.1 mol) of Et_3N and 0.6 g (4.91 mmol) of DMAP were added under Ar atmosphere. The reaction mixture was stirred under Ar atmosphere for 3 days. The CH_2Cl_2 solution was washed with 0.35 M $KHSO_4$ (3 × 50 mL) and water (3 × 50 mL). The solvent was evaporated under reduced pressure. The product **3** was purified by MPLC on RP18 to give 15.35 g (72%). ^1H NMR ($CDCl_3$) (δ ppm): 4.82 (m, 1 H), 3.66 (s, 3 H), 3.16 (s, 3 H), 2.47 (m, 3 H), 2.14 (m, 1 H), 1.50 (s, 9 H), 1.47 (s, 9 H), 1.45 (s, 9 H). MS (*m/z*): 447 (MH^+).
(22) Gellerman, G.; Elgavi, A.; Salitra, Y.; Kramer, M. *J. Peptide Res.* **2001**, *57*, 277.
(23) 5.11 g (13.2 mmol) of **4** and 2.28 g (13.2 mmol) of 2,4-dimethylphenyl hydrazine hydrochloride **5a** were refluxed in 100 mL of *iso*-propanol for 5 h. The reaction mixture was cooled to r.t. and the solvent was evaporated under vacuum to give 4.5 g of crude mixture. Yields (according to HPLC): 26.9% of mono-Boc product **6** and 50.5% of di-Boc product **7**. The mixture of **6** and **7** was dissolved in 40 mL dichloromethane. 100 μL of tri-*iso*-propyl silane and 40 mL of trifluoroacetic acid were added at 0 °C. The reaction mixture was stirred for 30 min at 0 °C and for 3 h at r.t. The solvent was evaporated and the residue was dissolved in 40 mL ACN/H_2O (2:1) and DIEA (to pH = 9). Fmoc-OSu (3.14 g) was then added and the final mixture was stirred overnight at r.t. The reaction mixture was acidified with 5% HCl. Acetonitrile was evaporated and the product was extracted with EtOAc (3 × 30 mL). The organic phase was washed with $NaHCO_3$ (3 × 30 mL), water and dried over $MgSO_4$. The pure product was isolated after elution from a self-packed column RP-MPLC (LiChroprep RP-18, 40-63 μL Merck) using acetonitrile and water (0.1% of TFA), 2.7 g (46%).
(24) (a) Commercially available 2,4-dimethylphenylhydrazine hydrochloride(**5a**) and 2,3-dimethylphenylhydrazine hydrochloride(**5c**) were purchased from Aldrich. (b) 2-Methyl-4-methoxyphenylhydrazine hydrochloride(**5b**) was synthesized according to the procedure from: Bare, T. M.; Chapdelaine, M. J.; Davenport, T. W.; Empfield, J. R.; James, R.; Garcia-Davenport, L. E.; Jackson, P. F.; McKinney, J. A.; McLaren, C. D.; Sparks, R. B. PCT Int. Appl., CODEN: PIXXD2 WO 9615127, **1996**. (c) 5-Ethyl-biphenyl-2-yl-hydrazine(**5d**) was prepared according to the procedure from: Katritzky, A.; Wang, Z. *J. Heterocycl. Chem.* **1988**, *25*, 671. (d) 5-Ethyl-biphenyl-2-ylamine was prepared according to procedure from: Bumagin, N. A.; Luzikova, E. V. *J. Organomet. Chem.* **1997**, *532*, 271.
(25) Selected data for compounds **8a** and **8d**. **8a**: ^1H NMR (d_6-DMSO) (δ ppm): 12.65 (br s, COOH), 10.66 (br s, NH), 7.87 (d, 2 H, *J* = 7.5 Hz), 7.66 (d, 2 H, *J* = 7.2 Hz), 7.62 (s, NH), 7.38–7.42 (m, 2 H), 7.26–7.33 (m, 2 H), 7.14 (s, 1 H), 7.12 (d, 1 H, *J* = 1.8 Hz), 6.70 (s, 1 H), 4.18–4.21 (m, 4 H), 3.12–3.18 (m, 1 H), 2.96–3.01 (m, 1 H), 2.38 (s, 3 H), 2.33 (s, 3 H). HRMS (*m/z*): 455.1964 (MH^+, calculated 455.1971 for $C_{28}H_{27}N_2O_4$). **8d**: ^1H NMR (d_6-DMSO) (δ ppm): 12.75 (br s, COOH), 10.65 (br s, NH), 7.87 (d, 2 H, *J* = 7.5 Hz), 7.60–7.75 (m, 5 H), 7.51 (t, 2 H, *J* = 7.5Hz), 7.37–7.40 (m, 4 H), 7.20–7.34 (m, 2 H), 7.17 (d, 1 H), 6.98 (d, 1 H, *J* = 1.2 Hz), 4.15–4.28 (m, 4 H), 3.19–3.25 (m, 1 H), 3.00–3.15 (m, 1 H), 2.72 (q, 2 H, *J* = 7.5 Hz), 1.28 (t, 3 H, *J* = 7.5Hz). HRMS (*m/z*): 531.2281 (MH^+, calculated 531.2284 for $C_{34}H_{31}N_2O_4$).
(26) Selected data for compound **19**: ^1H NMR (d_6-DMSO) (δ ppm): 12.50 (br s, COOH), 10.66 (br s, NH), 7.10 (s, 1 H), 7.07 (d, NH), 6.92 (d, 1 H, *J* = 7.8 Hz), 6.69 (s, 1 H), 4.09–4.18 (m, 1 H), 3.04–3.11 (m, 1 H), 2.87–2.95 (m, 1 H), 2.36 (s, 3 H), 2.34 (s, 3 H), 1.33 (s, 9 H). HRMS (*m/z*): 333.1831 (MH^+, calculated 333.1814 for $C_{18}H_{25}N_2O_4$).