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Synthesis of (*S*)-5,6-dibromo-tryptophan derivatives as building blocks for peptide chemistry

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ABSTRACT

5,6-Dibromo-tryptophan is an interesting amino acid whose derivatives and analogues are found in a variety of highly bioactive natural compounds. Notwithstanding its relevance no data concerning this compound are found in the literature. Here an efficient pathway for the synthesis of 5,6-dibromo-tryptophan derivatives is reported. The reaction is performed by using 6-Br-isatin as starting material. Selective bromination at position 5 was followed by BH_3 reduction of the intermediate α-keto-amide and alkylation with Ser-OH in Ac_2O/AcOH. Optical resolution was effected by enzymatic de-acetylation of the obtained racemic mixture. Finally, in situ N^{α}-Boc protection of the optically pure *S* form yielded the desired N^{α}-Boc-(*S*)-5,6-dibromo-tryptophan.

1. Introduction

In a large number of biochemical processes brought about by functional proteins the presence of the essential amino acid tryptophan plays a crucial role. The indolic side chain of this residue is in fact unique for structural and chemical properties. Its planar and heteroaromatic moiety is the largest among the naturally occurring amino acids and possesses electrostatic and amphiphilic properties capable of highly influencing the protein function. The usual H-bonding activity is accompanied by more specific interactions connected with the presence of the electron-rich indole nucleus and this property is responsible for the interaromatic ring-stacking effects and cation–π interactions which often control protein conformation and enzymatic activity.[1–3]

In accordance with the above reported multifunctional character tryptophan represents a target substrate for both synthetic and biosynthetic transformations in search of lead compounds and building blocks for pharmaceutical applications.[4a,b] Literature examination shows that halogenated derivatives and in particular brominated indole derivatives have attracted the attention of both chemists and biologists.[5–7] The presence of halogen atoms in the indole nucleus may in fact significantly influence bioactivity and bioavailability[5] of bioactive compounds offering, at the same time, suitable models for SAR studies and selective functionalization strategies through coupling reactions mediated by the presence of the aromatic bromine atoms. Methods for efficient synthesis of tryptophan analogues with monobromo- or dibromo-substitution on the indole ring are therefore highly desirable.

Representative examples of natural compounds related to 5,6-dibromo-tryptophan as the bioprecursor and possessing interesting biological activity, often associated with unusual structural features, are reported in Figure 1.

A wide range of indoles[13] and tryptophan derivatives[14] have been previously synthesized by using enzymatic and chemical methods. When enzymes are involved[14a] the accessibility of the enzyme active site may be sensibly restricted by substituents on the tryptophan indole ring. Substitution at the 4- or 7-positions leads to poor substrates while substituents at the 5- or 6-positions are generally accepted with the large iodo or nitro groups usually poorly tolerated.[14c] Concerning the series of halogenated tryptophans, an examination of the literature shows that, whereas some monohalo-tryptophans have recently been described,[14a–g] synthesis and property of dihalo-tryptophans are not known.

Taking into account the low availability of marine organisms which are the main source of dihalo-tryptophan containing molecules as well as the potential of dihalo-tryptophans as building blocks for the chemistry of peptides, we report here synthesis and the properties of 5,6-dibromo-tryptophan (di-BrTrp) derivatives.

2. Chemistry

The adopted synthetic protocol is reported in Scheme 1. Selective bromination of 6-bromo-isatin was performed by following

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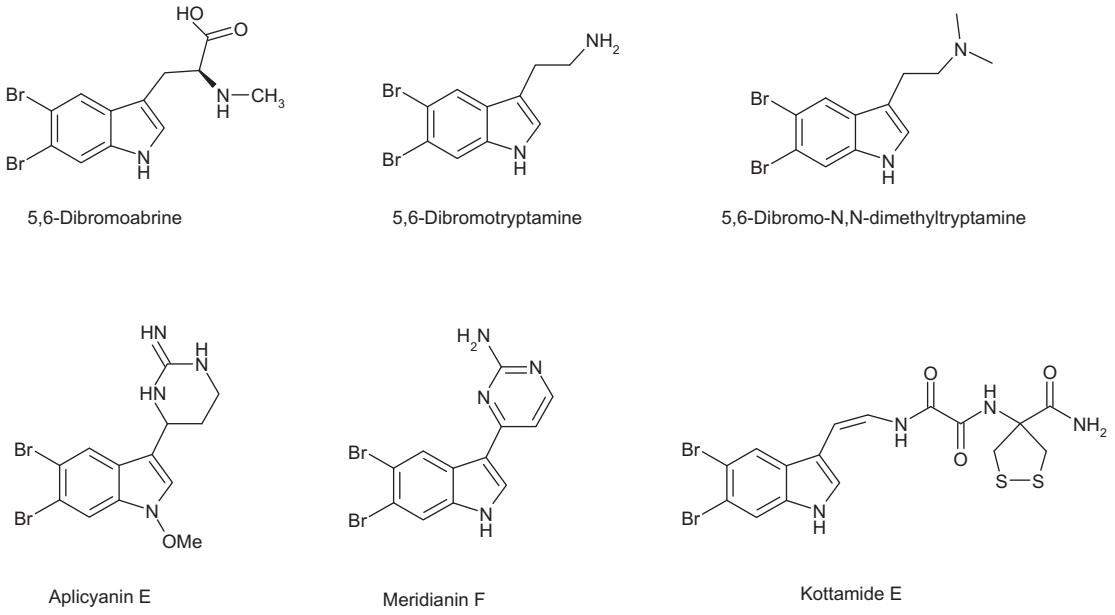
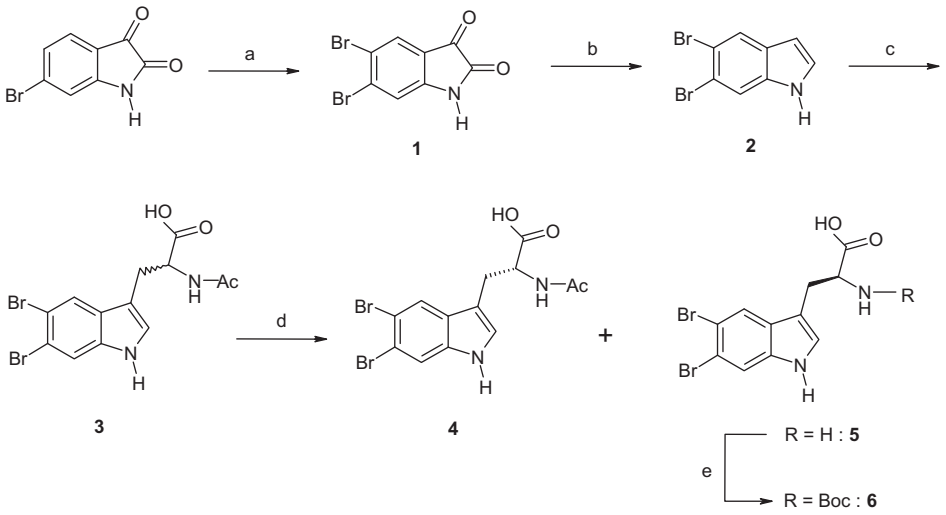


Figure 1. Literature examples of 5,6-dibromoindole containing natural products: 5,6-dibromo-abrine (see Ref. 8); 5,6-dibromo-tryptamine (see Refs. 8,9); 5,6-dibromo-*N*,*N*-dimethyltryptamine (see Ref. 9); aplicyanin E (isolated from the ascidians *Aplidium cyaneum* and characterized by cytotoxic activity on the human tumour cells and antimitotic activity; see Ref. 10a,c); meridianin F (isolated from the tunicate *Aplidium meridianum*; see Refs. 10b,11); kottamide E (from ascidian *Pycnoclavella kottae*; see Refs. 12a,b).



Scheme 1. Synthesis of N^{α}-Ac-5,6-(*R*)-di-BrTrp-OH **4** and N^{α}-Boc-(*S*)-5,6-di-BrTrp-OH **6**. Reagents and conditions: (a) Br_2, THF, AcOH, reflux, 48 h, 57%; (b) 1 M BH_3·THF, THF, rt, 3 h, 68%; (c) (*S*)-Ser-OH, Ac_2O, AcOH, under N_2 atm, reflux, 3.5 h, 50%; (d) amano acylase, $CoCl_2$·6 H_2O, phosphate buffer at pH 8, 37 °C, 6 h; (e) Boc_2O, 10% NaOH, H_2O/dioxane 1:3, rt, 5 h, 23% from **3**.

the procedure described by Vine et al.[15] 5,6-Dibromo-isatin (**1**)[16] was carefully purified by multiple crystallization from AcOH and then reduced by a solution of BH_3 in THF to give 5,6-dibromo-indole (**2**) in good yield (68%).[17] As previously reported, attempts to obtain **2** by using $LiAlH_4$ or $NaBH_4$ were unsuccessful while reduction with $LiBH_4$ in THF gave only very low yields.[18] Synthesis of the 5,6-dibromo-tryptophan derivative (**3**) was performed by exploiting the nucleophilic reactivity of the indole ring at the C-3 carbon atom. Thus, 5,6-dibromo-indole (**2**) was reacted with (*S*)-serine in a mixture of acetic acid/acetic anhydride to give in good yields the desired *N*-acetyl-(*R*,*S*)-5,6-dibromo-tryptophan (**3**).[19] The reaction proceeds through *N*-acetyl-α,β-unsaturated species as electrophilic intermediates[14c,d] and this leads, as expected, to complete loss of the (*S*)-serine chiral centre. 'Amano' acylase, an enzyme commonly used for optical resolutions of racemic *N*-acetyl amino acids was used to resolve the racemic *N*-acyl derivative **3**. As previously reported[14c] the two large substituents at the 5- and 6-positions of the indole ring sensibly slow down the enzymatic hydrolysis rate as compared with the *N*-acetyl-(*R*,*S*)-tryptophan. Thus, in order to obtain in one step the (*S*)-form as derivative suitable for peptide synthesis, the usually reported procedures[14c,e] have been modified. The aqueous buffered solution was acidified (pH 3) and extracted with EtOAc to give *N*-acetyl-(*R*)-5,6-dibromo-tryptophan (**4**) ca. 90% ee. Subsequent evaporation of the aqueous layer and treatment of the residue with *tert*-butyl dicarbonate (Boc_2O) gave the desired enantiopure *N*-Boc-(*S*)-5,6-dibromo-tryptophan (**6**), ca. 82% ee.[20] The enantiomeric excess of products (**4**) and (**6**) was tested by the HPLC

method reported by Jin et al.[21] Chromatography was performed at room temperature on a polysaccaride-derived chiral stationary phase (CSP) covalently bonded on silica matrix. The packing composition of the used column (Chiralpak IA) is amylose tris(3,5-dimethylphenylcarbamate) immobilized on 5 μm silica-gel,[22] a chiral selector system which shows high enantioselectivity for the resolution of *N*-Boc-α-amino acids and their esters.[21]

3. Conclusion

In conclusion, an efficient synthesis of the *N*-Boc 5,6-dibromo-(*S*)-tryptophan and *N*-acetyl 5,6-dibromo-(*R*)-tryptophan, useful and still unknown building blocks for both peptide chemistry and synthesis of natural products, has been developed by using a combined chemical and enzymatic approach.

Supplementary data

Supplementary data (^{1}H NMR spectra, ^{13}C NMR spectra, chiral HPLC runs are reported in Supplementary data) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.041.

References and notes

1. Horovitz, A.; Fersht, A. R. *J. Mol. Biol.* **1990**, *214*, 613–617.
2. Black, K. M.; Clark-Lewis, I.; Wallace, C. A. *Biochem. J.* **2001**, *359*, 715–720.
3. Sanderson, J. M. *Org. Biomol. Chem.* **2005**, *3*, 201–212.
4. (a) Sawyer, T. K. In *Structure Based Drug Design: Disease, Targets, Techniques and Development*; Veerapandian, P., Ed.; Marcel Dekker: New York, 1997; (b) Gordon, E. M. In *Combinatorial Chemistry and Molecular Diversity in Drug Discovery*; Gordon, E. M., Kerwin, J. F., Jr., Eds.; Wiley-Liss: New York, 1998.
5. Neidleman, S. L.; Geigert, J. *Biohalogenation: Principles, Basic roles and Applications*; Ellis Horwood Ltd: Chichester, 1986.
6. Lindquist, N.; Fenical, W.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 2303–2304.
7. Yeh, E.; Garneau, S.; Walsh, C. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3960–3965.
8. Tasdemir, D.; Bugni, T. S.; Mangalindan, G. C.; Concepción, G. P.; Harper, M. K.; Ireland, C. M. *Z. Naturforsch. Sect. C* **2002**, *57*, 914–922.
9. Djura, P. S.; Sullivan, D. B.; Faulkner, D. J.; Arnold, E. C.; Clardy, J. J. *J. Org. Chem.* **1980**, *45*, 1435–1441.
10. (a) Reyes, F.; Fernández, R.; Rodríguez, A.; Francesch, A.; Taboada, S.; Avila, C.; Cuevas, C. *Tetrahedron* **2008**, *64*, 5119–5123; (b) Walker, S. R.; Carter, E. J.; Huff, B. C.; Morris, J. C. *Chem. Rev.* **2009**, *109*, 3080–3098; (c) Šíša, M.; Pla, D.; Altuna, M.; Francesch, A.; Cuevas, C.; Albericio, F.; Álvarez, M. *J. Med. Chem.* **2009**, *52*, 6217–6223.
11. Hernández Franco, L. H.; Bal de Kier Joffé, E.; Puricelli, L.; Tatian, M.; Seldes, A. M.; Palermo, J. A. *J. Nat. Prod.* **1998**, *61*, 1130–1132.
12. (a) Appleton, D. R.; Copp, B. R. *Tetrahedron Lett.* **2003**, *44*, 8963–8965; (b) Appleton, D. R.; Page, M. J.; Lambert, G.; Berridge, M. V.; Copp, B. R. *J. Org. Chem.* **2002**, *67*, 5402–5404.
13. (a) Da Settimo, A.; Nannipieri, E. *J. Org. Chem.* **1970**, *35*, 2546–2551; (b) Jiang, B.; Smallheer, J. M.; Amaral-Ly, C.; Wuonola, M. A. *J. Org. Chem.* **1994**, *59*, 6823–6827.
14. (a) Goss, R. J. M.; Newill, P. L. A. *Chem. Commun.* **2006**, *47*, 4924–4925; (b) Angelini, E.; Balsamini, C.; Bartoccini, F.; Lucarini, S.; Piersanti, G. *J. Org. Chem.* **2008**, *73*, 5654–5657; (c) Blaser, G.; Sanderson, J. M.; Batsanov, A. S.; Howard, J. A. K. *Tetrahedron Lett.* **2008**, *49*, 2795–2798; (d) Yokoyama, Y.; Nakakoshi, M.; Okuno, H.; Sakamoto, Y.; Sakurai, S. *Mag. Res. Chem.* **2010**, *48*, 811–817; (e) Konda-Yamada, Y.; Okada, C.; Yoshida, K.; Umeda, Y.; Arima, S.; Sato, N.; Kai, T.; Takayanagi, H.; Harigaya, Y. *Tetrahedron* **2002**, *58*, 7851–7861; (f) Oikawa, Y.; Hirasawa, H.; Yonemitsu, O. *Chem. Pharm. Bull.* **1982**, *30*, 7437; (g) Bittner, S.; Scherzer, R.; Harlev, E. *Amino Acids* **2007**, *33*, 19–42; (h) Sui, Y.; Liu, L.; Zhao, J. L.; Wang, D.; Chen, Y. J. *Tetrahedron Lett.* **2007**, *48*, 3779–3782; (i) Park, K.; Gopalsamy, A.; Aplasca, A.; Ellingboe, J. W.; Xu, W.; Zhang, Y.; Levin, J. I. *Bioorg. Med. Chem.* **2009**, *17*, 3857–3865.
15. Vine, K. L.; Locke, J. M.; Ranson, M.; Pyne, S. G.; Bremner, J. B. *Bioorg. Med. Chem.* **2007**, *15*, 931–938.
16. *Procedure for preparation of **1**:* To a mixture of 6-bromoisatin (0.88 mmol) in AcOH (9 ml), Br_2 (0.88 mmol) was added and the mixture was stirred for 48 h at reflux. After this time the reaction was cooled at 0 °C, and the solid residue was paper filtered off, washed with AcOH and dried in the oven. The crude product was then recrystallized from AcOH to give the pure orange coloured product **1**, 57%; mp >270 °C with decomposition. ^{1}H NMR, 300 MHz, DMSO-d_6 δ = 7.23 (1H, s, H^7); 7.82 (1H, s, C^4), 11.22 (1H, brs, NH). ^{13}C NMR, 75 MHz, DMSO-d_6 δ = 117.40, 117.64, 119.61, 129.33, 133.81, 150.67, 159.83, 183.12.
17. *Procedure for preparation of **2**:* To a solution of **1** (0.327) in THF (10 ml) a solution of 1 M BH_3·THF (2.5 equiv) was added dropwise at 0 °C, then the mixture was stirred at rt for 3 h. After this time, the reaction was quenched by slowly adding 2 ml of demineralized water, then 2N HCl was added to pH 3. THF was removed under reduced pressure and the aqueous residue extracted with two portions of EtOAc. The organic layers were washed with two portions of brine, dried over Na_2SO_4 and evaporated under reduced pressure to give a yellow oil. The crude product was purified by silica gel chromatography (petroleum benzene/Et_2O 9:1) to give the pure product **2** as a greyish crystalline solid, mp = 154–155 °C, Rf = 0.2 (Et_2O/petroleum ether 1:5), 68%. ^{1}H NMR, 300 MHz, DMSO-d_6 δ = 6.42 (1H, m, H^3), 7.42 (1H, t, H^2), 7.75 (1H, s, H^7), 7.93 (1H, s, H^4), 11.35 (1H, brs, NH). ^{13}C NMR, 75 MHz, DMSO-d_6 δ = 101.53, 113.62, 115.45, 116.71, 124.96, 128.76, 129.44, 136.35.
18. (a) Tsintsadze, T. G.; Khoshtariya, T. E.; Kurkovskaya, L. N.; Mirziashvili, N. T.; Sikharulidze, M. I. *Chem. Heterocycl. Compd.* **2002**, *38*, 472–476; (b) Khoshtariya, T. E.; Matnadze, M. M.; Mirziashvili, N. T.; Kurkovskaya, L. N.; Sikharulidze, M. I.; Dzhashi, T. O. *Chem. Heterocycl. Compd.* **2004**, *40*, 1454–1459.
19. *Procedure for preparation of **3**:* To a mixture of **2** (0.363 mmol) in Ac_2O (0.31 ml) and AcOH (0.7 ml) was added (*S*)-Ser-OH (0.72 mmol) under reflux. The reaction mixture was stirred for 3 h at reflux under N_2 atmosphere. Then the reaction was allowed to rt and the pH was adjusted at 11 by adding a few drops of 30% NaOH solution. The solvent was then removed under reduced pressure and the residue taken up in demineralized water. The aqueous layer was extracted with two portions of Et_2O and then the pH was adjusted to 3 by adding 5% HCl. The aqueous layer was extracted with EtOAc and the organic phase was dried on Na_2SO_4, evaporated under reduced pressure to give a crude **3**. This was purified by silica gel column chromatography (EtOAc/AcOH 99:1 to EtOAc/AcOH 97:3) to give product **3** as an oil, Rf = 0.4 (EtOAc/AcOH 9:1), 50%. ^{1}H NMR data, and MS are consistent with the enantiopure product **4**.
20. *Typical procedure:* **3** (0.254 mmol) was dissolved in a 3.1 ml of phosphate buffer pH 8 (Sigma Aldrich) containing $CoCl_2$ (3.9×10^{-4} mmol) and the mixture was added to a mixture of Amano Acylase (103 mg) in phosphate buffer (10.3 ml) at 37 °C for 24 h. Then the mixture was concentrated under reduced pressure and the pH adjusted to 3 by adding 2N HCl. The suspension was extracted by two portions of EtOAc and the organic layer dried on Na_2SO_4, filtered and evaporated under reduced pressure to give the crude product **4** as a colourless oil. The aqueous layer was dried under reduced pressure and the crude residue, containing product **5** and the buffer residues, was dissolved in dioxane/H_2O 13:1 (14 ml) then 10% NaOH (0.96 ml) and Boc_2O (1.9 mmol) were added. The mixture was stirred for 5 h at rt, then solvent was removed under reduced pressure and the crude residue was taken up in H_2O and the pH adjusted to 9 by 10% NaOH and extracted with Et_2O. The aqueous phase was then acidified to pH 3 with 2N HCl and extracted with EtOAc. The organic layer was dried on Na_2SO_4, filtered and evaporated under vacuum to give the crude **6**. The residue was purified by RP–HPLC C18 95:5–10:90 H_2O/CH_3CN, Rt = 16.6 min to give the pure **6** as a colourless oil, overall yield 23%. Compound **4**: ^{1}H NMR, 300 MHz, DMSO-d_6 δ = 1.75 (3H, s, CH_3CO), 3.07 (2H, m, βCH_2), 4.29 (1H, m, αCH), 7.18 (1H, s, H^2), 7.69(1H, s, H^7), 7.88 (1H, s, H^4), 7.96 (1H, d, NHCO), 11.13 (1H, s, indolic NH). ^{13}C NMR, 75 MHz, DMSO-d_6 δ = 23.06, 27.46, 53.62, 110.81, 113.32, 115.49, 116.74, 123.48, 125.10, 129.07, 136.49, 169.87, 173.95; MS (ES−): 403.0 [M−H]$^-$. $[\alpha]_D^{20}$: −13.7, *c* = 1 (DMF). Compound **6**: ^{1}H NMR, 300 MHz, DMSO-d_6 δ = 1.37 (9H, s, Boc), 3.15 (2H, m, βCH_2), 4.38 (1H, s, αCH); 5.55 (1H, d, Boc-NH); 7.04 (1H, s, H^2), 7.61 (1H, s, H^7), 7.78 (1H, s, H^4), 10.62 (1H, s, indolic NH). ^{13}C NMR, 75 MHz, 10% DMSO-d_6 in $CDCl_3$ δ = 27.45, 28.32, 54.13, 79.13, 109.54, 113.43, 115.64, 116.09, 123.00, 125.84, 128.77, 136.09, 155.13, 173.64; MS (ES+): 485.1 [M+Na]$^+$; $[\alpha]_D^{20}$: −2.5, *c* = 1 (DMF).
21. Jin, J. Y.; Bae, S. K.; Lee, W. *Chirality* **2009**, *21*, 871–877.
22. *Chromatographic conditions*. Column: Chiralpak IA 250 mm L × 4.6 mm I.D. Conditions: detection UV at 220 nm; flow rate: 0.9 ml/min; injection of 20 μL of a solution containing 1 mg/mL of analyte; isocratic elution with a mixture of hexane/2-propanol/TFA (85:15:0.1) in 30 min, for chiral HPLC runs of product **3**, **4** and **6** (further details in Supplementary data).