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Two New Protecting Groups for the Guanidino Function of Arginine

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In Memory of Esteemed Prof. Dr. h. c. mult. Salimuzzaman Siddiqui FRS.

Abstract. Two new synthons, Fmoc-L-Arg(biphenyl-4-sulphonyl)-OH (**8**) and Fmoc-Arg(4-methoxy-3-t-butylbenzenesulphonyl)-OH (**14**), are prepared for the synthesis of arginine-containing peptides. These groups are cleaved by commonly employed trifluoroacetic acid and methanesulphonic acid. Kinetic studies reveal that extended bicyclic aromatic conjugation, as in biphenyl, slightly improves the acid lability compared to the electron-donating t-butyl group.

The trifunctional guanidino side chain of arginine **1** has to be protected during peptide synthesis or otherwise intramolecular cyclization is observed. The search for mild cleaveable side chain arginine protecting groups is still one of the challenging problems in current peptide chemistry, though various approaches are described in the literature including protection by (i) nitro, (ii) urethane, (iii) trityl or (iv) arylsulphonyl groups [1].

Classical blocking of the guanidino function by the nitro group [2] often results in the formation of undesirable lactam formation [1,3]. The application of t-butyloxycarbonyl (Boc) and bis-adamantyloxycarbonyl $(Adoc)_2$ groups as arginine side chain protecting groups are examples of the utilization of urethane residues used for the solid phase peptide synthesis of longer chain peptides as demonstrated in the literature [4–6]. The most serious disadvantages of these groups arise from incomplete masking of the guanidino function which results in the formation of considerable amounts of ornithine-containing peptides [7]. Further efforts in this direction led to the use of the well known acid labile trityl group for the blocking of the N^{ω} nitrogen of the guanidino function, but side chain acylation is not completely prevented [8] and the synthon Fmoc-Arg(Trt) has low solubility in the coupling solvents used in solid phase peptide synthesis, and often long coupling times are observed [9].

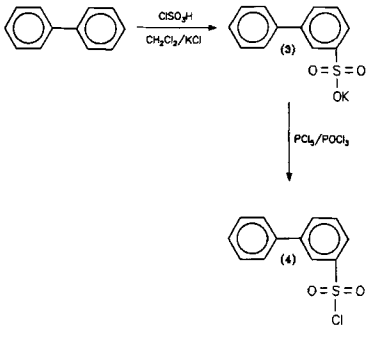
In recent years much efforts have been put towards the development of mild, acid labile arylsulphonyl-type protecting groups eliminating side reactions. The originally proposed p-toluenesulphonyl group [10] suffers shortcomings like lactam formation [1] or removal under drastic conditions [1,10]. Several efforts were made to incorporate various electron-donating substituents to the benzenesulphonyl group which resulted in several new guanidino protecting groups. The relative acid lability of these groups is in the following order: 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) > 4-methoxy-2,6-dimethylbenzenesulphonyl (Mds) = 2,4,6-trimethoxybenzenesulphonyl (Mtb) > 2,3,4,5,6-pentamethylbenzenesulphonyl (Pme) > 4-methoxy-2,3,5,6-tetramethylbenzenesulphonyl (Mte) > mesitylene-2-sulphonyl (Mts) = p-methoxybenzenesulphonyl (Mbs) > 2-methoxy-4,6-di-methylbenzenesulphonyl (iMds) [11]. Among these various multi-substituted benzenesulphonyl protecting groups, the 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr) residue, developed by Fujino *et. al.* [11], is the most acid labile group in this class. Fmoc-Arg(Mtr)-OH (**2**) is successfully used in solid phase peptide synthesis and various challenging multiple arginine-containing residues were synthesized [12–16]. Unfortunately, the removal of Mtr requires relatively strong conditions compared to reagents applied in Fmoc-based syntheses [17–20]

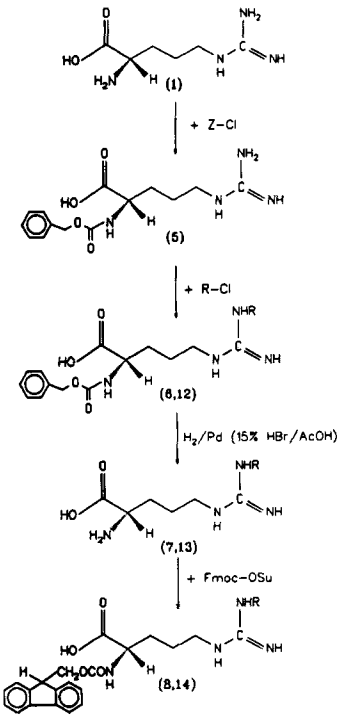
and thus can result in the modification of tryptophan [20].

The search for a more acid labile guanidino protecting group over Mtr resulted in the development of the 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) group [21]. This TFA labile group is successfully employed in solid phase peptide synthesis [17]. However, stronger acidolytic removal [18,19] and unexpected cleavage in multiple arginine fragments, resulting in sulphonated arginine residues [18], suggest that both, Mtr and Pmc groups, are not fully satisfactory. In view of these drawbacks, it is still worthwhile working on the rational design of benzenesulphonyl based N^G-protecting groups causing less side effects and enhanced acid lability compared to the conventional ones. In this paper we report the synthesis of two new N^G-protected Fmoc derivatives of L-arginine, namely Fmoc-L-Arg(biphenyl-4-sulphonyl)-OH (Fmoc-L-Arg(Bip)-OH) **8** and Fmoc-L-Arg(4-methoxy-3-t-butylbenzenesulphonyl)-OH (Fmoc-L-Arg(Tbs)-OH) **14**, and the study of the kinetics of acid hydrolysis.

Results and Discussion

Biphenyl is used as starting material for the synthesis of the first protecting group biphenyl-4-sulphonyl (bip). The sulphonation of biphenyl with chlorosulphonic acid and subsequent work up leads to biphenyl-4-sulphonic acid and the addition of a saturated KCl solution affords the biphenyl-4-sulphonic acid potassium salt **3**. Compound **3** is converted into biphenyl-4-sulphonyl chloride **4** by reacting it with PCl_5 and $POCl_3$ (Scheme 1). N^α-benzyloxycarbonyl-L-arginine **5** is prepared by the reaction of benzylchloroformate [22] with L-arginine at pH 9–10. This derivative is coupled with **4** in THF at pH 12–13 to achieve N^α-benzyloxycarbonyl-N^G-biphenylsulphonyl-L-arginine **6**. The N^α-benzyloxycarbonyl group is removed by catalytic hydrogenation with 10 % Pd/C in glacial acetic acid to afford N^G-biphenylsulphonyl-L-arginine acetate **7**. Finally, the treatment of **7** with 9-fluorenylmethyloxycarbonylsuccinimide according to Tesser *et. al.* [23] results in the required Fmoc derivative **8** (Scheme 2). The purity of the product is checked





R = Biphenyl-4-sulphonyl (Bip), 4-Methoxy-3-t- butyl-benzenesulphonyl (Tbs)

by TLC and HPLC (Figure 1) and the structure is confirmed by FDMS and elemental analysis.

The second protecting group, 4-methoxy-3-t-butylbenzenesulphonyl (Tbs), is synthesized from 2,4-di-t-butylphenol. To increase the acid lability, the hydroxyl group is converted to the electron-donating methoxy group by treating the corresponding phenol with potassium-t-butoxide and methyl iodide, whereby 2,4-di-t-butylanisole **9** is obtained. Sulphonation is carried out with chlorosulphonic acid to obtain 4-methoxy-3-t-butylbenzenesulphonic acid potassium salt **10** followed by chlorination with PCl_5 and $POCl_3$, affording 4-methoxy-3-t-butylbenzenesulphonyl chloride **11** (Scheme 3). Its mass spectrum shows a molecular ion peak at m/z 262 indicating the elimination of one of the t-butyl groups. This fact is also consistant with the elemental analysis. The position of the sulphonyl residue is assigned by ^1H NMR. The downfield double doublet at δ 7.68 (J = 8.77, 2.48 Hz) shows ortho- and meta-couplings, suggesting that an electron-withdrawing group in immediate vicinity is attached to C-6-H. The upfield doublet at δ 7.05 shows an ortho coupling (J = 8.78 Hz) assigned to C-5-H, while another downfield doublet at δ 7.92 shows only a small meta coupling constant (J = 2.49 Hz), attributed to C-2-H. Treatment of **5** with **11** in THF at pH 14 results in N^α-benzyloxycarbonyl-4-methoxy-3-t-butylbenzenesulphonyl-L-arginine **12** (Scheme 2). The benzyloxycarbonyl group is removed by 15 % HBr/acetic acid to afford N^G-4-methoxy-3-t-butylbenzenesulphonyl-L-arginine hydrobromide **13**. Finally, the required Fmoc-

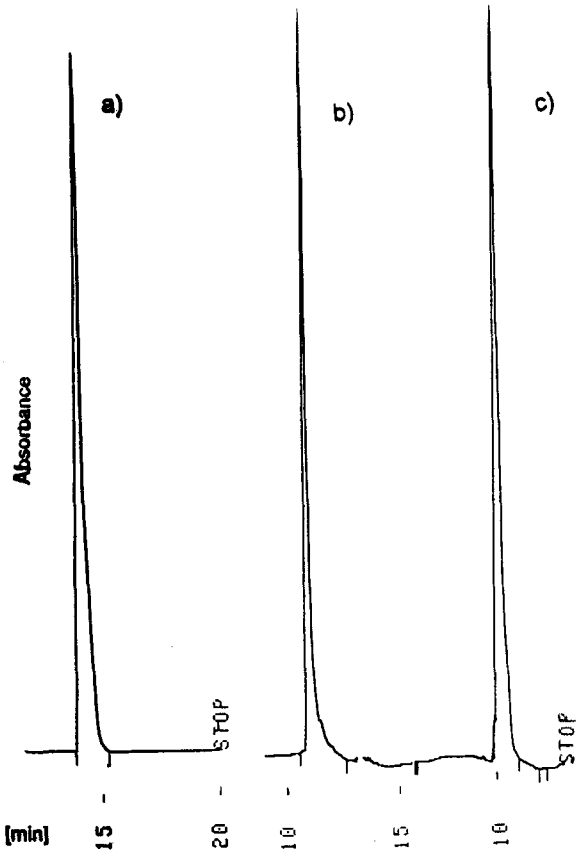


Fig. 1 Analytical HPLC of purified Fmoc-Arg derivatives. a: Fmoc-Arg(Mtr)-OH (**2**); b: Fmoc-Arg(Bip)-OH (**8**); c: Fmoc-Arg(Tbs)-OH (**14**). Chromatographic conditions: Nucleosil 120-5 C_{18} (Macherey-Nagel, 250/1/4"/4.6 μm); eluents: 40 % CH_3CN and 0.015 % TFA in water (A), 80 % CH_3CN and 0.015 % TFA in water (B); linear gradient from 0 to 100 % in 11 min; flow rate: 1.2 ml/min; concentration: 1 μg/μl; injected volume: 25 μl detection at 301 nm.

L-arginine derivative **14** is prepared by the action of 9-fluorenylmethyl-oxycarbonylsuccinimide on **13** according to the standard procedure [23]. The structures and purities are confirmed by mass spectroscopy, elemental analysis, TLC and HPLC (Figure 1).

These two model protecting groups are synthesized in order to observe the effect of acidolytic removal on extended bicyclic aromatic conjugated systems over an electron-donating t-butyl group in the benzenesulphonyl residue. For this purpose the Fmoc-L-arginine derivatives **8** and **14** were subjected to acidolytic cleavage under different concentrations of TFA and MSA (Table 1). In a preceeding communication [24] we investigated the acid lability of three other new substituted aromatic sulphonyl groups for the protection of the arginine side chain: the 2,4,6-triisopropylbenzenesulphonyl (Tip), 4-methoxy-3,5-di-t-butylbenzenesulphonyl (Mtbs) and phenanthrene-3-sulphonyl (Phen) residues, with the result that their acid lability is decreasing as follows: Mtr > Tip > Mtbs > Phen. From these investigations the conclusion can be drawn, that the effect of electron-donating substituents in increasing the acid lability of the arylsulphonyl residue seems to be of the order methyl > isopropyl > t-butyl. The kinetic studies (Figures 1-3, Table 1) of the present communication demonstrate that an extended bicyclic aromatic conjugated system as in biphenyl slightly improves the acid lability compared to the electron-donating t-butyl group at the meta position to the sulphonyl group. The results of these investigations are of enormous importance for the rational design of an easy accessible and at low cost produceable protecting group for the arginine side chain removable under mild conditions and causing no side reactions during peptide synthesis.

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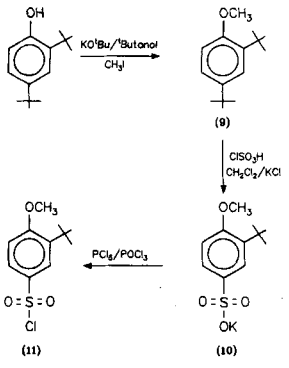

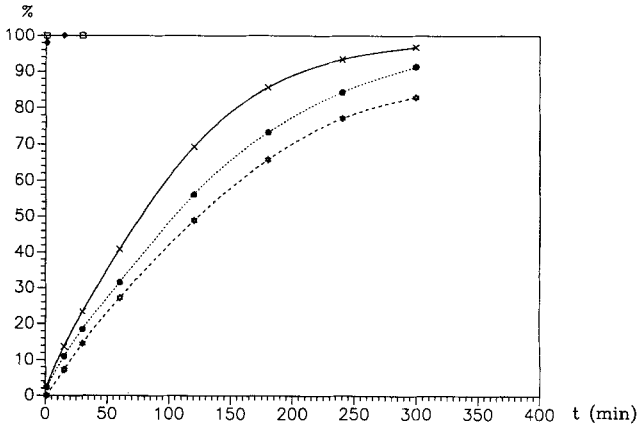

Fig. 2 Cleavage kinetics of the Mtr group from Fmoc-Arg(Mtr)-OH under the following acidolytic conditions: *–*–* TFA/anisole/water (90:5:5), •–•–• TFA/water (95:5); ×–×–× TFA/ethanedithiol/water (90:5:5); •–•–• TFA/MSA (90:10); □–□–□ TFA/MSA (80:20). For chromatographic conditions see legend to Figure 1, for kinetic investigations see experimental.

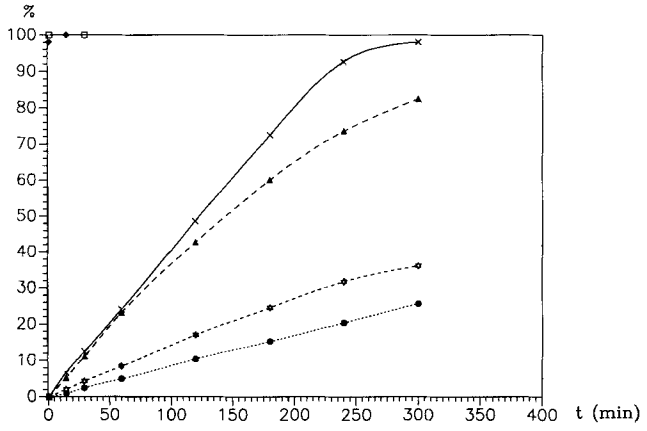

Fig. 3 Cleavage kinetics of the Mtr, Bip and Tbs groups from Fmoc-Arg derivatives under the following acidolytic solutions: Bip ×–×–× TFA/MSA (50:50); Tbs ▲–▲–▲ TFA/MSA (50:50); Bip ∗–∗–∗ TFA/MSA (80:20); Tbs •–•–• TFA/MSA (80:20); Mtr •–•–• TFA/MSA (90:10); Mtr □–□–□ TFA/MSA (80:20). For chromatographic conditions see legend to Figure 1, for kinetic investigations see experimental.

Experimental

Solvents were purified and dried in the usual way. The boiling range of the petroleum ether used was 35–65 °C. N-Ethyldiisopropylamine (b.p 126–127 °C) was dried over CaH_2 and stored over KOH pellets. Melting points were determined using a Büchi appratus (model 510, Switzerland) and are uncorrected. Optical rotation values were measured with a Carl-Zeiss polarimeter 370748 (Germany), using a 1-dm cell. ^1H NMR and ^{13}C NMR spectra were recorded using a Bruker WM 250 instrument (solvent: $CDCl_3$, DMSO-d_6 and tetramethylsilane (TMS) as an internal standard). Electron impact (EI) and field desorption mass spectroscopy (FD-MS) were performed using a Varian MAT 711 mass spectrometer. Column chromatography was carried out on silica gel 60 (0.063–0.200 μm, Merck), aluminium oxide (basic, Macherey-Nagel; Düren) and silica gel for flash chromatography (4 mm, J. T. Baker). Thin layer chromatography (TLC) was recorded using pre-coated glass plates silica gel 60 F_{254}, layer thickness 0.2 mm (Merck, Darmstadt). Indication was performed using UV absorption (254), followed by development of the chromatogram with ninhydrin, chlorine/o-tolidine or iodine vapours. Analytical HPLC was performed on an Eppendorf-Biotronik (Maintal) apparatus consisting of HPLC pumps (model BT 8100), a Rheodyne (USA) injection system (model 7125), a UV detector (BT 8200), connected with a Shimadzu (C-R6A) chromatopac printer and the analytical runs were done on a Nucleosil column 120-5C_{18} ($250/_{1/4}$" /4.6 μm; Macherey-Nagel; Düren) with a flow rate of 1.2 ml/min. The following gradient was applied, 0–100 B within 11 min, A = 40 % CH_3CN and 0.015 % TFA in H_2O, B = 80 % and 0.015 % TFA in H_2O (detection at l = 301 nm). A Knick pH-meter was used for pH measurments.

Biphenyl-4-sulphonic acid potassium salt (**3**)

Concentrated sulphuric acid (50 g, 0.50 mol) was added to biphenyl (50 g, 0.32 mol). The reaction mixture was heated at 70 °C for 12 h on water bath. The colour of the solution changed to pink. The sulphonated material was diluted with 150 ml water. The colour gradually changed to yellow and finally to white. After removing unreacted biphenyl by filtration, cupric oxide (12.91 g, 0.162 mol) in 100 ml water was added. The sparingly soluble copper salt of monosulphonic acid then seperated out. The crude salt was recrystallized from hot water and dried over P_2O_5 in vacuo. After dissolving in hot water, hydrogen sulphide gas was passed thoroughly until all copper was removed as copper sulphide. The clear filtrate was concentrated and 100 ml saturated solution of KCl was added to obtain the biphenyl-4-sulphonic acid potassium salt which was filtered and recrystallized from hot water to afford 42.1 g (47.7 %) of **3**.

Table 1 Deprotection of N^G-protected Fmoc-Arg derivatives under different acidolytic conditions

Protecting Group	Cleavage Reagent	Reaction Time (min) and Percentage Cleaved [%] 1	15	30	60	120	180	240	300
Mtr									
	a)	[0]	[7.20]	[14.54]	[27.21]	[48.88]	[65.75]	[77.21]	[82.99]
	b)	[2.33]	[10.99]	[18.51]	[31.61]	[56.09]	[73.31]	[84.38]	[91.32]
	c)	[2.84]	[13.70]	[23.39]	[40.92]	[69.30]	[85.64]	[93.45]	[96.82]
	d)	[98.01]	[100]						
	e)	[100]							
Bip									
	e)	[0]	[2.01]	[4.22]	[8.40]	[17.00]	[24.50]	[31.74]	[36.18]
	f)	[0]	[6.60]	[12.41]	[24.00]	[48.59]	[72.44]	[92.63]	[98.02]
Tbs									
	e)	[0]	[0.92]	[2.40]	[4.90]	[10.39]	[15.20]	[20.39]	[25.60]
	f)	[0]	[5.20]	[11.12]	[23.11]	[42.62]	[60.00]	[73.52]	[82.41]

a) = TFA : Ani : Water (90 : 5 : 5), b) = TFA : Water (95 : 5), c) = TFA : EDT : Water (90 : 5 : 5), d) = TFA : MSA (90 : 10), e) = TFA : MSA (80 : 20), f) = TFA : MSA (50 : 50)
TFA = Trifluoroacetic acid, Ani = Anisole, EDT = Ethanedithiol, MSA = Methanesulphonic acid

Biphenyl-4-sulphonyl chloride (**4**)

Biphenyl-4-sulphonic acid potassium salt (40 g, 146.86 mmol) was moistened with 5 ml $POCl_3$ and then mixed with PCl_5 (40 g, 192 mmol). The mixture was refluxed on an oil bath at 130 °C for 3 h and then 50 ml benzene was added. The mixture was distilled under vacuum and to the residue 200 ml diethyl ether was added. It was filtered and allowed to crystallize at 0 °C to afford 28.7 g (77.3 %) of **4**; mp. 106–108 °C; R_f = 0.26 (petroleum ether/dichloromethane, 8:2); EI-MS (rel. int.): m/z 252 (M^+, 42 %), 254 (M^+ + 2, 18 %), 153 (M^+ – 99, 100 %).

$C_{12}H_9O_2SCl$	Calcd: C 57.03	H 3.59	S 12.68	Cl 14.02
(252.72)	Found: C 57.03	H 3.42	S 12.70	Cl 14.34

N^{α}-Benzyloxycarbonyl-L-arginine (Z-L-Arg-OH (**5**)

L-Arginine (30 g, 172 mmol) was suspended in 0.5 N 150 ml of NaOH cooled to 0 °C and under continuous stirring benzyl chloroformate (25 ml, 176.29 mmol) was carefully added to adjust the pH between 9–10. After the addition of benzyl chloroformate was complete, the reaction mixture was stirred for further 2 h at 0 °C and the pH allowed to decrease to 7.0–7.5 and this value was maintained till the reaction was complete. The precipitate was filtered, washed with water, recrystallized from hot water (70 °C) and dried in high vacuum to afford 42.5 g (80 %) of **5**; mp. 184 °C (dec.); $[\alpha]_D^{22}$ = –9.3° (c 2, 1N HCl); R_f = 0.3 ($CHCl_3/CH_3OH/C_6H_6/H_2O/(CH_3)_2CO$, 8:8:8:1:1.5); FD-MS: m/z 308.4 (M^+).

$C_{14}H_{20}N_4O_4$	Calcd: C 54.53	H 6.53	N 18.17
(308.33)	Found: C 54.46	H 6.76	N 18.01

N^{α}-Benzyloxycarbonyl-N^{G}-biphenyl-4-sulphonyl-L-arginine (Z-L-Arg(Bip)-OH (**6**)

To a continuously stirred suspension of **5** (10 g, 32.43 mmol) in 100 ml of THF, 4 N NaOH solution was added until the pH attained a value of 14. The resulting solution was cooled to 0 °C and 4 (14 g, 55.39 mmol), dissolved in 25 ml THF was added dropwise in such a way that the value of pH remained between 12–13 and the temperature did not increase above 5 °C. The reaction mixture was stirred at 0 °C for 2 h and for further 3 h at room temperature. The resulting solution was diluted with cold water and neutralized with 3 N HCl and concentrated on a rotary evaporator till all the THF was removed. The remaining aqueous solution was acidified to pH 3-4 by the addition of a 4 N citric acid solution and extracted with ethyl acetate. The organic phase was washed several times with water, dried over anhydrous sodium sulphate and concentrated in vacuo to yield 12.67 g (74.5 %) of **6**; mp. 152–153 °C; $[\alpha]_D^{22}$ = –4o (c 1, DMF); R_f = 0.25 ($CHCl_3/CH_3OH/C_6H_6/H_2O/(CH_3)_2CO$, 8:8:8:1:1.5); FD-MS: m/z 525.2 (M^+ + 1).

$C_{26}H_{28}N_4O_6S$	Calcd: C 59.52	H 5.38	N 10.67	S 6.11
(524.6)	Found: C 59.69	H 5.53	N 10.79	S 6.03

N^{G}-Biphenyl-4-sulphonyl-L-arginine acetate (H-L-Arg(Bip)-OH. HOAc (**7**)

A solution of **6** (5.56 g, 1048 mmol) in 100 ml glacial acetic acid was hydrogenated for 6 h over 10 % Pd/C (0.5 g), whereby the reaction was completed. The reaction mixture was filtered through a bed of Celite, toluene was added and then the mixture evaporated *in vacuo* till all the acetic acid was removed. The residue was further dried with an oil pump to afford 3.9 g (83 %) of **7**; mp. 152–153 °C; $[\alpha]_D^{22}$ = –2° (c 1, DMF); R_f = 0.25 (n-butanol/acetic acid/water, 3:1:1); FD-MS: m/z 450.2 (M^+).

$C_{20}H_{26}N_4O_6S$	Calcd: C 53.32	H 5.81	N 12.43	S 7.11
(450.51)	Found: C 52.80	H 5.67	N 12.01	S 7.05

N^{α}-9-Fluorenylmethyloxycarbonyl-N^{G}-biphenyl-4-sulphonyl-L-arginine (Fmoc-L-Arg (Bip)-OH (**8**)

To a solution of **7** (5.09 g, 11.29 mmol) in 50 ml acetonitrile/water 60:40 freshly distilled triethyl amine was added to adjust the pH between 8-9. The solution was cooled to 0 °C and a solution of Fmoc-OSu (4.5 g, 13.33 mmol) in 25 ml acetonitrile was added to it in one portion. More triethyl amine was added to maintain the pH at 8.5. The mixture was stirred for 15 min at 0 °C and 1 h at room temperature, whereby the reaction was complete. The solution was concentrated *in vacuo*, 3 N 100 ml citric acid was added to the residue, and then it was extracted with ethyl acetate. The organic phase was washed thoroughly with water, dried over anhydrous sodium sulphate and concentrated in vacuo, to yield 4.68 g (67.6 %) of **8**; mp. 118–120 °C; $[\alpha]_D^{22}$ = –2.25° (c 1, DMF); R_f = 0.25 ($CHCl_3/CH_3OH/C_6H_6/H_2O$, 8:8:8:1); FD-MS: m/z 613.2 (M^+ + 1).

$C_{33}H_{32}O_6N_4S$	Calcd: C 64.69	H 5.26	N 9.14	S 5.23
(612.71)	Found: C 64.33	H 5.31	N 8.90	S 5.66

2,4-Di-t-butylanisole (**9**)

2,4-Di-t-butylphenol (10 g, 48.46 mmol) was dissolved in t-butanol (50 ml) and potassium t-butoxide (8 g, 71.28 mmol) in 30 ml t-butanol was added with constant stirring under argon at room temperature. Freshly distilled methyl iodide (6 ml, 95.94 mmol) was gradually added to the above solution and the reaction was allowed to complete. After 5 h the reaction mixture was poured in water and extracted with petroleum ether. The extract was washed several times with water and dried over sodium sulfate. Removal of the petroleum ether afforded **9** as light yellow oil 9.7 g, (90.9 %); R_f = 0.45 (petroleum ether).

$C_{15}H_{24}O$	Calcd: C 81.76	H 10.98
(220.35)	Found: C 81.25	H 10.88

^1H NMR ($CDCl_3$): δ [ppm]: 1.49 (s, 9H, *o*-$C(CH_3)_3$), 1.58 (s, 9H, *p*-$C(CH_3)_3$), 3.97 (s, 3H, OCH_3), 6.99 (d, J = 8.4 Hz, 1H C-3-H), 7.3-7.8 (two multiplets, 1H, C-5-H), 7.51 (br. s, 1H, C-6-H); EI-MS (rel. int.): m/z 262 (M^+, 19 %); 247 (M^+ + 15, 100 %).

4-Methoxy-3-t-butylbenzenesulphonic Acid Potassium Salt (**10**)

To the cold solution of **9** (9.5 g, 43.11 mmol) in 50 ml dichloromethane, chlorosulphonic acid (3.4 ml, 51.06 mmol) was dropwise added. The reaction mixture was stirred for 1 h at 0 °C and further 30 min at room temperature. After this period, 100 ml water was added and dichloromethane was removed under reduced pressure. Then a 25 ml saturated KCl solution was added to obtain the title compound. Yield 7.6 g (62.4 %).

4-Methoxy-3-t-butylbenzenesulphonyl Chloride (**11**)

compound **10** (7.5 g, 26.55 mmol) was moistened with 3 ml $POCl_3$ and then PCl_5 (8 g, 38.41 mmol) was added. The reaction was subjected to the usual work up used for the prepa-

ration of compound **4** and 6.1 g (87.5 %) of **11** was obtained; mp. 76-77 °C; R_f = 0.13 (petroleum ether/dichloromethane, 8:2).

$C_{11}H_{15}O_3SCl$ (262.75) Calcd: C 50.28 H 5.75
Found: C 49.84 H 5.75

^1H NMR ($CDCl_3$): δ [ppm]: 1.32 (s, 9H, *o*-$C(CH_3)_3$), 4.02 (s, 3H, *p*-$C(CH_3)_3$), 7.04 (d, J = 8.7 Hz, 1H, C-5-H), 7.69 (dd, J = 2.48, 8.77 Hz, 1H, C-6-H), 7.92 (d, J = 2.49 Hz, 1H, C-2-H); FD-MS: m/z 262.2 (M^+), 247 (M^+ - 15).

N^a-Benzyloxycarbonyl-N^G-4-methoxy-3-t-butylbenzenesulphonyl-L-arginine (Z-L-Arg(Tbs)-OH (**12**)

Compound **5** (4.7 g, 15.24 mmol) was suspended in 40 ml THF and then basified with a 4 N NaOH solution till the pH attained the value of 14. The reaction mixture was cooled to 0 °C and then 4-methoxy-3-t-butylbenzenesulphonyl chloride (6.0 g, 22.83 mmol), dissolved in 30 ml THF, was added dropwise to this mixture. The reaction was worked up as for compound **5** to afford **12** as amorphous solid. Yield 4.6 g (55.6 %); mp. 158–160 °C; $[\alpha]_D^{22}$ = –0.85° (c 2, DMF); R_f = 0.30 ($CHCl_3/CH_3OH/C_6H_6/H_2O/(CH_3)_2CO$, 8:8:8:1:1.5); FD-MS: m/z = 535.3 (M^+).

$C_{25}H_{34}N_4O_7S$ (534.64) Calcd: C 56.16 H 6.41 N 10.47
Found: C 55.73 H 6.61 N 9.10

N^G-4-Methoxy-3-t-butylbenzenesulphonyl-L-arginine hydrobromide (H-L-Arg(Tbs)-OH. HBr (**13**))

Compound **12** (4.5 g, 8.41 mmol) was dissolved in a solution of 15 % HBr/acetic acid (15 ml). After 30 min the reaction mixture was worked up as for compound **7** to afford compound **13** (3.1 g, 77.5 %); mp. 158–160 °C; FD-MS m/z = 481.4 (M^+).

$C_{17}H_{29}N_4O_5SBr$ (481.42) Cd: C 43.87 H 6.28 N 12.03 S 6.88 Br 17.16
Fd: C 43.67 H 6.19 N 11.84 S 6.70 Br 18.91

N^a-9-Fluorenylmethyloxycarbonyl-N^G-4-methoxy-3-t-butylbenzenesulphonyl-L-arginine (Fmoc-L-Arg(Tbs)-OH (**14**))

Compound **13** (3.0 g, 6.23 mmol) was dissolved in 25 ml acetonitrile/water 1:1 and the solution basified with triethyl amine till the value of pH was 8–8.5. Fmoc-OSu (2.5 g, 7.41 mmol), dissolved in 10 ml acetonitrile, was added in one portion, and the reaction was worked-up as usual. The corresponding Fmoc-arginine derivative **14** was obtained as amorphous solid (2 g, 51.5 %); mp. 174–176 °C; $[\alpha]_D^{22}$ = –4.25° (c 2, DMF); R_f = 0.21 ($CHCl_3/CH_3OH/C_6H_6/H_2O/(CH_3)_2CO$, 8:8:8:1:1.5); FD-MS m/z = 623.7 (M^+).

$C_{32}H_{38}N_4O_7S$ (622.74) Calcd: C 61.71 H 6.15 N 8.99 S 5.14
Found: C 63.07 H 5.93 N 6.68 S 5.80

Kinetic Investigations

5 mg Fmoc-L-Arg(Mts) **2** and the corresponding Bip **8** and Tbs **14** derivatives were dissolved in the systems a–f each (see Table 1). 50 ml aliquots were drawn after 1, 15,30, 60, 120, 180, 240 and 300 min intervals and then 1000 µl buffer A (40 % CH_3CN and 0.015 % TFA in water) was added. In case of the systems d, e and f, the pH was maintained at 3–4 by the addition of N-ethyldiisopropylamine (ca. 101–102 µl). Finally 300 µl were injected. For chromatographic conditions see legends to the figures 2 and 3.

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