Mild Reductive Cleavage of the 9-Anthracenesulfonamido Function. The 9-Anthracenesulfonyl (Ans) Group: A New Approach to the Protection of the Guanidino Function of Arginine Residues in Peptide Synthesis

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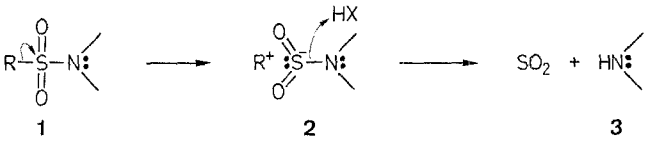
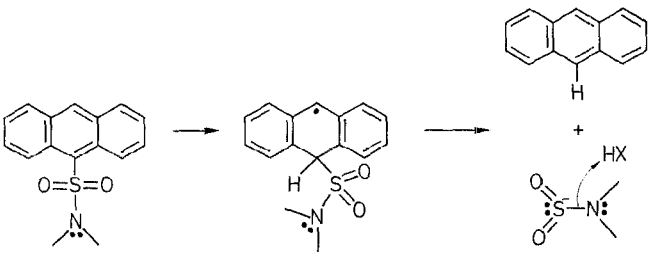
Convenient syntheses of L-arginine derivatives bearing the 9-anthracenesulfonyl (Ans) protective group at the ω-guanidino site are described. Simple arginine-containing peptides bearing the Ans group are shown to be stable to the normal conditions of peptide synthesis. The Ans group is removed either by acidolysis with trifluoroacetic acid over a period of hours or by a variety of novel, mild reducing conditions including reaction with aluminum amalgam and photo-induced ruthenium-catalyzed reduction with 1-benzyl-1,4-dihydronicotinamide. Application of the Ans group to a synthesis of bradykinin is reported, and detailed preparative procedures for the useful derivatives N^{α}-Boc-L-Arg(N^{ω}-Ans)-OH and N^{α}-Bpoc-L-Arg(N^{ω}-Ans)-OH are described.

Arenesulfonamides have had a long and honorable service as protected derivatives of amines.[1] The conventional means of cleaving a sulfonamido group have involved acidolysis of the N–S bond or its reductive cleavage by dissolving metal or solvated electron-type agents.[2] Although attachment of electron-donating groups to the arene function allows tempering the harshness of the acidolysis conditions required for clean removal, the arenesulfonamides still must be classed among protective groups that can be cleaved only under rather forceful conditions. Little attention seems to have been given to means of achieving heterolytic cleavage of the C–S bond as indicated in the general conversion, **1** → **2** → **3**. Such cleavage might be achieved by appropriate modification of the carbon structure of an arene or alkanesulfonamide.



1 **2** **3**

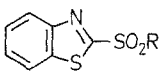
In this paper we report realization of reductive cleavages of the 9-anthracenesulfonamido function for the special case in which the nitrogen is part of a guanidino group. The ease of addition of electrophiles and radicals to the 9 and 10-positions of an anthracene moiety is well-known, and we reasoned that a wide variety of reagents should be capable of effecting multistep reductive cleavage to the aminosulfinate anion **2** via intermediates in which the equivalent of a hydrogen atom or a hydride



4 **5** **6**

anion has added to the 9-position, as in the hypothetical sequence **4** → **5** → **6**. The destabilizing effect of R–SO_2 substituents on sp^2 hybridized carbon is expected to facilitate attack at the 9 over the 10-site, and in a study of *ipso* attack by carbon radicals in the related system **7** the leaving group capacity of the R–SO_2 group has been shown to be high.[5]

As peptide synthesis has been extended to larger and more fragile target molecules the need has grown for protective functions that are completely resistant to conventional operations of synthesis, purification, and storage, yet are selectively and quantitatively cleavable under mild conditions.[3] Our application of the 9-anthracenesulfonamide group to the protection of the guanidino function of arginine addresses a major problem of modern peptide synthesis, for none of the traditional means of protection is free from complications, which include failure to mask the intramolecular nucleophilic reactivity of the guanidino ε-nitrogens, enhancement of the electrophilic reactivity of the guanidino carbon, incomplete removal, and ornithine formation during removal. Thus, the ω-nitro group **8** is notorious for by-product formation during reductive removal,[6] the very bulky *bis*-Adoc group **9** is difficult to introduce and generates ornithine upon removal,[7] and the traditional *p*-toluenesulfonyl group **10** can be difficult to remove cleanly. Recently, benzenesulfonyl functions bearing multiple electron-donating substituents, **11** and **12**, have been found to have many attractive features including removal by acidolysis for several hours in trifluoroacetic acid (TFA).[8] However, even these conditions are too harsh for some of the more demanding synthetic applications.[9] The arginine guanidino function is a formidable challenge for the designer of new blocking groups, and it should be noted that the



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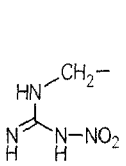
Ans group is unlikely to solve all of the above problems. Our aim in this study was the development of a protective group that could be compatible with the Fmoc and Bpoc/solid-phase strategies and that offers a range of conditions for its removal.



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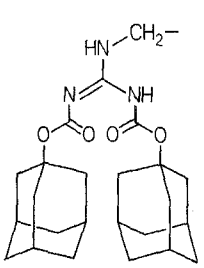

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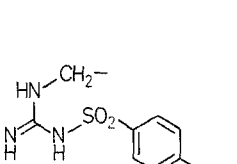

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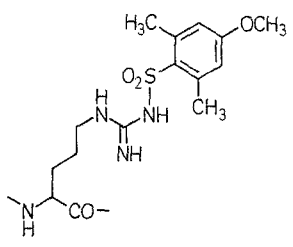

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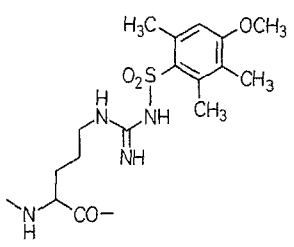

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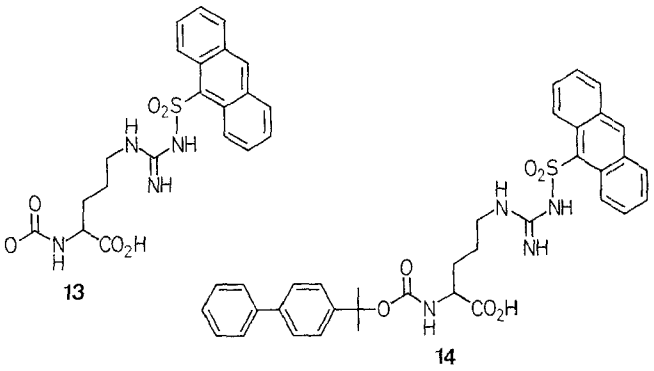
In this paper we report convenient syntheses of the useful synthetic intermediates N^{α}-Boc-L-Arg(N^{ω}-Ans)-OH(**13**) and N^{α}-Bpoc-L-Arg(N^{ω}-Ans)-OH (**14**) by conventional routes from 9-anthracenesulfonyl chloride (9-Ans-Cl). These derivatives are applied successfully to simple examples of both classical and solid-phase peptide synthesis, as indicated by the following results and yields: dicyclohexylcarbondiimide-(DCC)-hydroxybenzotriazole mediated[10] formation of N^{α}-Boc-L-Arg(N^{ω}-Ans)-L-Asp(OBzl)$_2$, 87%; N^{α}-Boc-L-Arg(N^{ω}-Ans)-L-Phe-NH(2-naphthyl), 75%; N^{α}-Boc-L-Arg(N^{ω}-Ans)-L-Leu-OMe, 90%; Boc-L-Phe-L-Arg(N$^{\omega}$-Ans)-L-Leu-OMe, 77%; solid-phase formation of a *bis*-Ans-blocked bradykinin: H-L-Arg(N^{ω}-Ans)-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg(N^{ω}-Ans)-OH using the Fmoc strategy for N-terminal amine protection, 45% after chromatographic purification). Finally, we demonstrate clean cleavage of the Ans function under the acidolytic conditions used with **11** and **12**, as well as under a variety of mild reductive conditions.



13 **14**

In a preliminary study, the formation of anthracene was used as a screen for the cleanness of removal of the Ans group from H-Arg (N^{ω}-Ans)-OH. Quantitative reduction was observed for the following reaction conditions: catalytic hydrogenation (H_2/Pd, 24 h); treatment with samarium iodide in THF/*t*-BuOH for 15 min;[11] reaction with aluminum amalgam in water, pH 7; and photolysis (dicyanobenzene sensitizer, 8 h) in the presence of any of the following hydrogen atom donors: sodium borohydride, sodium cyanoborohydride, triethylsilane, and 9,10-dihydroanthracene. Although many of these conditions can undoubtedly be applied to the deblocking or arginine-containing peptides, we have confined our attention to an acidolytic and two of the reductive conditions.

When Boc-L-Phe-L-Arg(N^{ω}-Ans)-L-Leu-OMe was allowed to stand at 25 °C for 5 h in trifluoroacetic acid containing anisole and thioanisole, a quantitative yield of the pure salt of H-L-Phe-L-Arg-L-Leu-OMe was isolated after evaporation and trituration with ether; the acidolytic lability of the Ans group is thus comparable to that observed for **12**. A 72% yield for the same tripeptide ester was obtained from a two-step cleavage procedure in which the Ans group is first removed by stirring at 25 °C for 1 h in tetrahydrofuran/water (9:1) with aluminum amalgam, and the Boc group is then cleaved, after evaporation of the filtrate, by brief treatment with trifluoroacetic acid. Application of the aluminum amalgam procedure to Z-L-Arg(N^{ω}-Ans)-OH resulted in recovery of Z-L-Arg-OH in 90% yield.

Exceptionally mild and clean photolytic cleavage of the Ans group is observed when an Ans-functionalized arginine-containing peptide is irradiated for 20 h at 25 °C with a tungsten lamp in methanol solution containing the hydride donor 1-benzyl-1,4-dihydronicotinamide (6 equiv) and *tris*-o, o'-bipyridylruthenium dichloride (0.5 equiv), as reported by Ohno, Tanada, and their collaborators for the reduction of alkyl halides and alkyl sulfonates.[12] Thus, clean and quantitative cleavage to anthracene and *N*-*t*-butoxycarbonyl-L-argininyl-L-phenylala-

ninyl-2-naphthylamide is seen when the above conditions are applied to Boc-L-Arg(N^{ω}-Ans)-L-Phe-NH-(2-naphthyl). Similar clean deblocking was observed for the *bis*-Ans derivative of bradykinin.

We have successfully used the Ans group in Bpoc-based, solid-phase syntheses of a series of peptides from the sequence of bovine pancreatic trypsin inhibitor. The following summarizes our experiences to date:

1. Like other arenesulfonyl functions, the Ans group does not completely mask neighboring group reactivity of the guanidino function toward activated acyl carbon. Thus, we have not been successful in acylations with symmetrical anhydrides of Ans-blocked Arg derivatives.

2. Like the Mtr group, the Ans group is too acid-labile to be used in Boc-based solid-phase syntheses.

3. The Ans group has been tailored to the needs of Fmoc or Bpoc-based solid-phase synthesis, but is too acid-labile to be suitable for Boc-based synthesis.

High resolution ^{1}H-NMR spectra were obtained on a Bruker WM-250 instrument. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tennessee. Amino acid analyses were conducted on a Glenco MM-60 micro-column analyzer; hydrolysates were prepared in evacuated Reacti-Therm tubes (Pierce) at 130 °C for 2 h in propionic acid-HCl. Analytical TLC was performed on glass precoated silica gel 60 plates (Merck F-254). Preprative layer chromatography was performed on Analtech GF 1,000 μ and GF 2,000 μ silica gel plates, and flash chromatography on silica gel 60 (230–400 mesh) using 100% CH_2Cl_2 as eluent. Preparative silica gel was Kieselgel 60 (0.040–0.063 mm). HPLC was performed on a Waters system consisting of two Model 6.000-A pumps, a model 680 automated gradient controller, a model U6K injector, a Model 440 dual channel UV detector (280, 254 nm), an extended wavelength module (229, 214 nm) and a Model 730 data module. HPLC runs were conducted in the reverse-phase mode on Whatman Partisil columns.

Amino derivatives used in solid phase synthesis were purchased from Chemalog. Solvents and reagents for solid-phase synthesis were purified by distillation.

Sodium 9-Anthracenesulfonate was prepared by reaction of anthracene with SO_3 in dioxane solution, following the procedure of Zorn, Hinterhofer, and Schindlbauer,[13] with the following modifications. The reaction was run on six times the indicated scale, and temperature control during the SO_3 addition was best achieved by adding the liquid trioxide by hand using 5 mL glass pipets. A fresh pipet was substituted as soon as polymeric trioxide appeared at the pipet tip, and the addition took approximately 30 min. After dilution of the reaction with water as described, the finely divided suspension of unreacted anthracene was removed by filtration through a pad of celite. The resulting product contained traces of positional isomers, detectable by ^{1}H-NMR. These were best removed during the recrystallization of N^{α}-Boc-L-Arg(N^{ω}-Ans)-OH.

9-Anthracenesulfonyl Chloride:[14]

Sodium 9-anthracenesulfonate (98.2 g, 0.35 mol) is crushed in a mortar and dried at 80 °C, under vacuum for at least 1 h, then suspended in PCl_3 (500 mL), stirred, and heated at reflux for 17 h under nitrogen, then cooled and evaporated at aspirator vacuum. The solid residue is suspended in CH_2Cl_2 (2 L), and the grass-green mixture is filtered through Celite. The filtrate is cautiously washed with a slurry of ice and water (2 × 2 L), then washed with cold 5% aq. $NaHCO_3$ (2 × 2 L), then again with water and dried ($MgSO_4$). Evaporation gave a yellow solid; yield: 45.8 g (44%); mp 133 °C.

The 9-anthracenesulfonyl chloride is used within a day in one of the following preparations. (The purity of the PCl_3 is a key to the success of this preparation). The product can be recrystallized from hot toluene, but it is better used directly after solution at 25 °C in reagent grade acetone (100 mL) and filtration.

^{1}H-NMR (60 MHz, $CDCl_3$/TMS): δ = 9.15 (d, 2 H, J = 8 Hz); 8.65 (s, 1 H); 7.32–8.03 (m, 6 H).

MS (70 eV): m/z = 276 (M^+), 212 ($M^+ - SO_2$).

N^{α}-benzyloxycarbonyl-N^{ω}-(9-anthracenesulfonyl)-L-arginine:

A solution of N^{α}-benzyloxycarbonyl-L-arginine (0.5 g, 1.6 mmol) in 2.5 M NaOH (3 mL) is diluted with acetone (15 mL), stirred, and cooled to 0 °C. A solution of 9-anthracenesulfonyl chloride (1.0 g, 3.6 mmol) in a minimum volume of acetone is added dropwise, and the resulting mixture is stirred at 0 °C for 2 h and at 25 °C for 2 h. The solution is acidified to pH 3 with citric acid, and the solvent is evaporated in vacuum. The residue is extracted with EtOAc, and the pooled extracts are washed with water, then with 5% aq. $NaHCO_3$ solution (3 × 10 mL). The pooled aqueous extracts are acidiefied to pH 3 (citric acid) and extracted with EtOAc. The extracts are washed with water, dried ($MgSO_4$), and evaporated to give 850 mg of a yellow solid that could be recrystallized from MeOH/water to give the title compound; yield: 650 mg (72%).

$C_{28}H_{28}N_4S \cdot \frac{1}{2} H_2O$	calc.	C 60.31	H 5.25	N 10.05	S 5.75
(556.6)	found	60.19	5.28	9.94	6.00

^{1}H-NMR (250 MHz, $CDCl_3$/TMS): δ = 12.5 (br s, 1 H); 9.45 (d, 2 H, J = 10 Hz); 8.85 (s, 1 H); 8.14 (d, 2 H, J = 8 Hz); 7.65, 7.54 (2t, 4 H, J = 10 Hz, 8 Hz); 7.37 (s, 5 H); 5.04 (s, 2 H); 3.82 (br s, 1 H).

N^{α}-t-butoxycarbonyl-N^{ω}-(9-anthracenesulfonyl)-L-arginine:[14]

A solution of N-t-butoxycarbonyl-L-arginine (7.5 g, 27.8 mmol) in acetone (150 mL) at 0 °C is treated with cold 3 M aq. NaOH (40 mL) with stirring, followed by the dropwise addition of freshly prepared 9-anthracenesulfonyl chloride (12.6 g, 45.6 mmol) in acetone (150–200 mL) over 2 h. The heterogeneous mixture is stirred for 2 h at 25 °C, then acidified to pH 3 with citric acid. The residue obtained after evaporation of the acetone is extracted with EtOAc, which is in turn washed twice with water (discarded) and with 5% aq. $NaHCO_3$ (2 × 100 mL). The combined extracts were acidified to pH 3 with citric acid, then extracted with three portions of EtOAc. The pooled extracts are washed with water, dried ($MgSO_4$), and evaporated to give cruce product (13.4 g) which shows evidence by HPLV and ^{1}H-NMR of impurities derived from positional isomers of the anthracenesulfonyl function. Recrystallization is carried out by solution product (13.4 g) in MeOH (45 mL) at 25 °C followed by dropwise addition with stirring of water (15–18 mL). Alternatively product (13.4 g) is dissolved in a minimum volume (25–30 mL) warm MeOH; the solution is warmed, filtered, cooled to 25 °C and then to 0 °C overnight. After two recrystallizations, the title compound is obtained, homogeneous by ^{1}H-NMR and HPLC; yield: 9.2 g (65%); mp 118 °C (shrink), 130 °C (dec.).

$C_{25}H_{30}N_4O_6S \cdot \frac{1}{2}H_2O$	calc.	C 57.35	H 5.97	N 10.70	S 6.12
(532.6)	found	57.47	6.04	10.80	5.99

^{1}H-NMR (250 MHz, $CDCl_3$/TMS): δ = 9.38 (d, 2 H, J = 8 Hz); 8.50 (s, 1 H); 7.91 (d, 2 H, J = 8 Hz); 7.50, 7.40 (2t, 4 H, J = 8 Hz, 8 Hz); 4.02 (br s, 1 H); 3.00 (br s, 2 H); 1.32 (s, 9 H).

N^{α}-1-(4-Biphenylyl)-1-methylethoxycarbonyl-N^{ω}-(9-anthracenesulfonyl)-L-arginine:[14]

A solution of twice-recrystallized N^{α}-t-butoxycarbonyl-N^{ω}-9-anthracenesulfonyl-L-arginine (1.1 g, 2.1 mmol) in cold TFA (10 mL) is after 5 min evaporated in vacuum, to give a red-brown oil, which is converted to a yellow powder by trituration with ether. The collected solid is washed with ether, then dried at 1 Torr for 2 h to give 1.05 g N^{α}-deblocked arginine. The solid is dissolved in MeOH (10 mL), followed by addition of a MeOH solution of Triton B (1 equiv). If necessary, further small amounts of triton B are added dropwise with stirring to bring all the solid into solution (up to 2 equiv total base). The solvent is evaporated, and traces are removed by two successive evaporations of DMF (5 mL) at 25 °C in vacuum. To the resulting red syrup is added a mixture of freshly prepared 1-(4-biphenylyl)-1-methylethoxycarbonyl azide[15] (670 mg, 2.4 mmol) and Et_3N (0.28 mL, 2 mmol) in DMF (3 mL). The resulting solution is heated at 40 °C with stirring for 1 h, then cooled and cautiously partitioned in a separatory funnel between water (50 mL) and sufficient ether to allow an organic phase to separate, which is discarded (this operation is prone to form emulsions). After addition of CH_2Cl_2 (300 mL), the two-phase cloudy suspension is acidified with citric acid to pH 3, and the layers are separated. The organic phase is combined with a further CH_2Cl_2 extract of the aqueous layer, and the pooled organic layers are washed with water (100 mL) and brine (2 × 100 mL), then dried ($MgSO_4$), and treated dropwise with dicyclohexylamine until the solution is basic to moist pH paper. The resultant solution is then concentrated in vacuum to an oil. Trituration with petroleum ether to remove excess amine and drying in vacuum gave 1.9 g of a crude yellow-brown product, which is purified by flash chromatography. A 5 cm column containing an 8 cm

length of silica gel is equilibrated with dicyclohexylamine by passage of a solution of MeOH (7.5 mL) and dicyclohexylamine (2 mL) in CH_2Cl_2 (500 mL) through the column until the initially translucent silica gel developed opacity throughout the entire column. During the equilibration, the effluent is recycled by the addition of additional dicyclohexylamine (2 g/500 mL). The crude salt is applied to the column in a minimum volume of the above eluent and eluted with the above mixture. After the first 100 mL of solvent, the composition is changed to MeOH/dicyclohexylamine/CH_2Cl_2, 10:1:250. Two highly colored impurities are eluted first: at ca. 100 mL and 600–650 mL solvent volumes. The product appears in the effluent after passage of 675 mL solvent and is contained in the next 700 mL of eluent. This fraction is evaporated, and the residue is triturated with ether/petroleum ether to give the dicyclohexylammonium salt of the title compound as a yellow powder, homogeneous by HPLC and NMR; yield: 1.42 g (89 %); mp 115 °C (shrink), 121–125 °C (dec.).

$C_{48}H_{59}N_5O_6S$	calc.	C 69.12	H 7.13	N 8.40	S 3.84
(834.1)	found	69.46	7.14	8.55	4.02

^{1}H-NMR (250 MHz, CD_2Cl_2, TMS): δ = 9.50 (b s, 2 H); 8.52 (s, 1 H); 7.97 (d, 2 H, J = 8 Hz); 7.58–7.34 (13 H, m).

Methyl *N-t*-Butoxycarbonyl-N-(9-anthracenesulfonyl)-L-argininyl-L-leucinate: N^{α}-Boc-L-Arg(N^{ω}-Ans)-L-Leu-OMe:

A sample of Nα-Boc-L-Arg(N^{ω}-Ans)-OH · H_2O (300 mg, 0.573 mmol) is dried by three solutions and evaporations in dry MeCN, then added to (5 mL) containing H-L-Leu-OMe · HCl (105 mg, 0.578 mmol) and Et_3N (0.081 mL). With stirring at 0 °C 1-hydroxy-1*H*-benzotriazole (40 mg) and DCC (116 mg, 0.562 mmol) is added. After 40 h at 0 °C, the mixture is filtered, and the filtrate evaporated. A solution of the residue in EtOAc is extracted with 1 N HCl, water, 5 % aq. $NaHCO_3$, water, and brine, then dried ($MgSO_4$) and evaporated to give crude product (350 mg), which is purified by preparative layer chromatography to give the title compound, homogeneous by TLC and HPLC; yield: 325 mg (90 %).

$C_{32}H_{43}N_5O_7S$	calc.	C 59.89	H 6.75	N 10.91	S 5.00
(641.8)	found	60.10	7.00	10.83	5.23

^{1}H-NMR (250 MHz, $CDCl_3$/TMS): δ = 9.48 (d, 2 H, J = 10 Hz); 8.58 (s, 1 H); 7.96 (d, 2 H, J = 8 Hz); 7.55, 7.44 (2t, 4 H); 3.64 (s, 3 H); 1.34 (s, 9 H); 0.82 (dd, 6 H, J = 10 Hz, 8 Hz).

Application of a similar procedure to N^{α}-Boc-L-Arg(N^{α}-Ans)-OH and H-L-Asp(OBzl)$_2$ gave *N^{α}-Boc-L-Arg(N^{ω}-Ans)-L-Asp(OBzl)$_2$* in 87 % yield.

$C_{43}H_{47}N_3O_9S$	calc.	C 63.77	H 5.85	N 8.65	S 3.96
(781.9)	found	63.59	6.00	8.44	4.13

Methyl *N-t*-butoxycarbonyl-L-phenylalaninyl-*N*-(9-anthracenesulfonyl)-L-argininyl-L-leucinate: Boc-L-Phe-L-Arg-(N^{ω}-Ans)-L-Leu-OMe:

A solution of *N*-Boc-L-Arg(N^{ω}-Ans)-L-Leu-OMe (300 mg, 0.47 mmol) in trifluoroacetic acid (TFA; 10 mL) evaporated after 10 min at 25 °C. The residue is triturated with ether (2×), dried in vacuum for a few hours, dissolved in MeCN (5 mL), and chilled to 0 °C. To this solution is added with stirring Et_3N (0.066 mL, 0.47 mmol), 1-hydroxy-1*H*-benzotriazole (35 mg), Boc-L-Phe-OH (125 mg, 0.47 mmol), and DCC (95 mg, 0.46 mmol). After 2 h the solution is warmed to 25 °C overnight, then filtered and evaporated. A solution of the residue in EtOAc is washed successively with 1 M HCl, water, 5 % aq. $NaHCO_3$, and brine, then dried ($MgSO_4$), and evaporated to yield a residue (388 mg), which is purified by preparative layer chromatography (silica gel, EtOAc/toluene, 70:30) to give the desired product as a solid; yield: 280 mg (77 %).

$C_{41}H_{52}N_6O_8S$	calc.	C 62.42	H 6.64	N 10.65	S 4.06
(789.0)	found	62.32	6.50	10.41	4.40

^{1}H-NMR (250 MHz, $CDCl_3$/TMS): δ = 9.46 (d, 2 H, J = 10 Hz); 8.54 (s, H); 7.95 (d, 2 H, J = 8 Hz); 7.54, 7.42 (2t, 4 H, J = 10 Hz, 8 Hz); 7.13 (m, 5 H); 3.66 (s, 3 H); 1.27 (s, 9 H); 0.82 (d, 6 H).

Synthesis of Bradykinin Using L-Arg(N^{ω}-Ans) Derivatives:

The solid-phase peptide synthesis is carried out in a reaction vessel described by Merrifield,[16] and completeness of acylation steps is assayed by the qualitative ninhydrin procedure.[17] The synthesis is conducted on a *p*-(hydroxymethylphenoxy-functionalized resin obtained from Chemalog (Cat. # 57-8620-80); the hydroxyl function of the resin is esterified with N^{α}-Bpoc-L-Arg(*N*-Ans)-OH [dry resin (2.1 g/g) in CH_2Cl_2/DMF, 4:1 (25 mL) using DCC (700 mg) and 4-dimethylaminopyridine (375 mg), 5 °C → 25 °C for 5 h], following the procedure of Wang and Merrifield.[18] A loading level of 0.18 meq/g of resin is determined by amino acid analysis of a sample of resin using an external standard. Following the standard protocol of Chang and Meienhofer[19] deblockings are carried out with TFA for Bpoc and 50 % piperidine in CH_2Cl_2 for Fmoc. With the single exception of N^{α}-Boc-L-Arg(N^{ω}-Ans)-OH, acylations are carried out using symmetrical anhydrides prepared immediately before use. Acylations were conducted with the following series of protected amine acids: Fmoc-L-Phe-OH (sites 2 and 5), Fmoc-L-Pro-OH (sites, 3, 8, and 9), *N*-Fmoc-L-Ser(O^t*Bu*)-*OH (site 4)*, *Fmoc-Gly-OH (site 6)*, N^{α}-Boc-L-Arg(N$^{\omega}$-Ans)-OH (site 14). The last acylation is carried out using equal amounts of DCC and the blocked arginine derivative. Removal of the nonapeptide from the resin is carried out with 55 % TFA in CH_2Cl_2 containing 1 % anisole and 1 % 2-mercaptoethanol for 15 min at 25 °C. Evaporation and trituration of the residue with ether gave crude H-L-Arg(N^{ω}-Ans)-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg(N^{ω}-Ans)-OH, which is purified by chromatography on silica gel (60 g, pretreated with $ClSiMe_2(CH_2)_7CH_3$, then with $ClSiMe_3$, gradient elution with MeOH/HOAc, 99:1 to 7:3. By HPLC analysis, the product had a retention time of 11.5 min on a C8 reverse phase column (1 % HOAC in MeOH). A second chromatography on Sephadex LH 20 (1 % TFA in DMF) gave 136 mg protected bradykinin from the original 1 g of resin; yield: 45 %.

Amino acid analysis: Pro 2.89 (3), Phe 2.24 (2), Arg 2.0 (2), Gly 1.27 (1), Ser 0.63 (1). Amino acid analysis of a bradykinin sample from Vega: Pro 1.97 (3), Phe 1.98 (2), Arg 2.0 (2), Gly 1.11 (1), Ser 0.51 (1).

The protected nonapeptide (20 mg) is treated with TFA (1 mL) for 14 h, followed by evaporation, washing with ether and i-PrOH to give *bradykinin*; yield: 13 mg (77 %).

By HPLC analysis [214 nm detector, C8 reverse phase column, 17 % acetonitrile – 83 % phosphate buffer (0.15 M triethylaminephosphoric acid, pH 3.2)] the sample is 94 % pure with the same retention time as the reference.

The ^{1}H-NMR spectrum of the isolated material corresponds to that of the reference sample obtained from Vega.

General Procedures for Cleavage of the Ans Group:

Method A. Trifluoroacetic Acid: The peptide is dissolved in anhydrous TFA (1–2 % solution) containing 1 % of anisole and thioanisole. After 12–14 h at 25 °C the acid is evaporated, and the residue is washed with ether.

Method B. Aluminum Amalgam: The peptide is dissolved in THF-water, 9:1, and treated with freshly prepared amalgamated aluminum foil (3 mg/μmol peptide) and stirred at 25 °C for 1 h. The solution is filtered, and the collected precipitate washed with additional solvent. Evaporation gave the deblocked product.

Method C. 1-Benzyl-4-dihydronicotinamide: The peptide is dissolved in MeOH containing a 4-fold-excess of 1-benzyl-1,4-dihydronicotinamide and 0.5 equiv of Ru(bipyridine)$_3Cl_2 \cdot 6H_2O$. The pH is brought to 7 with 3 M NaOH and the solution is degassed and irradiated under nitrogen with a tungsten lamp for 20 hr. Evaporation gives the deblocked peptide, separated from the by-products by extraction or chromatography.

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