Synthesis of the Novel Amino Acid 4-Amino-3-(aminomethyl)benzoic Acid (AmAbz) and Its Protected Derivatives as Building Blocks for Pseudopeptide Synthesis

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4-Amino-3-(aminomethyl)benzoic acid (**1**) (AmAbz) is a novel unnatural amino acid with promise in applications as a building block for the synthesis of peptidomimetics and as a scaffold for combinatorial chemistry. It was efficiently synthesized in three steps (63% overall yield) from 4-aminobenzoic acid, by means of regioselective amidomethylation with hydroxymethylphthalimide. AmAbz (**1**) contains three distinct functionalities which could be discriminated from one another. Firstly, Boc_2O or Fmoc–OSu reacted selectively with the benzylamino group to give the monoprotected derivatives AmAbz(Boc) (**8a**) or AmAbz(Fmoc) (**8b**), respectively. The absence of acylation at the arylamino group was also noticed in coupling experiments using the BOP reagent and building block **8b**. This made protection of the arylamino group unnecessary either for peptide bond formation at the carboxyl group, or for subsequent elongation of a peptide chain at the benzylamino group. Finally, the arylamino group could be acylated under base-free, carbodiimide-mediated coupling conditions. These properties are illustrated by the solid-phase synthesis of the AmAbz-containing branched pseudopeptide Fmoc–Ala–Phe–AmAbz(H–Lys–Leu)–Val–Gly–NH_2 (**15**). The synthesis of Fmoc–AmAbz(Boc) (**10**) is also described.

Introduction

The availability of new tools and building blocks both for combinatorial organic chemistry and for peptidomimetic construction is of considerable interest in drug design.[1−3] In this context, rigid equivalents of dipeptides with distinguishable functional groups are promising targets. 4-Amino-3-(aminomethyl)benzoic acid (**1**) (AmAbz)[4] fulfils these criteria, but surprisingly, despite its simple structure, its synthesis and reactivity have not yet been investigated.[5] We propose here a synthetic route to this new amino acid. Pertinent features of AmAbz are: (i) the aromatic cyclic system, which is useful for mimicking a dipeptide residue exhibiting strongly reduced conformational mobility, (ii) two amino groups with a large difference in basicities, allowing the preparation of cyclic or branched peptide analogues, and (iii) a scaffold structure permitting presentation of three independent sets of building blocks, with potential applications in combinatorial chemistry.[3,6] Reported syntheses of compounds containing the AmAbz core deal exclusively with *N*-alkyl derivatives and involve multi-step reactions through nucleophilic substitution on halomethyl aromatic precursors,[7] intramolecular sulfonylamidomethylation of 4-aminobenzoic acid derivatives[8] or Friedel−Crafts acylation of an *o*-aminobenzylamine cyclic urea.[9] Here we describe a straightforward and efficient synthesis of AmAbz, starting from 4-aminobenzoic acid (**2**) and introducing the aminomethyl group onto the aromatic ring by amidomethylation.[10] The preparation of some AmAbz-derived building blocks designed for applications in solution or solid-phase synthesis is also described, as well as their reactivities in solid-phase elongation of branched peptides.

Results and Discussion

Synthesis of AmAbz (1)

The aminomethyl group was introduced at the position *ortho* to the arylamino group after conversion of 4-aminobenzoic acid (**2**) into the methylcarbamate derivative **3** (Scheme 1). Protection of the arylamino group was required because amidomethylation was performed, as usual,[10] in strong acid. Under these conditions, the unprotected arylamino group would be fully protonated, leading to substitution at the undesired *meta* position.[11] We selected a urethane-type protection which, compared with acyl protection, increases both the rates of electrophilic aromatic substitutions and their regioselectivities for the *ortho* position,[11,12] thanks probably to the more difficult protonation of the carbamate group.[13] The favourable *meta*-directing effect of the carboxyl group present in reactant **3** was also expected to increase the regioselectivity towards the right position.

Carbamate **3** was prepared in high yield (97%) by treating 4-aminobenzoic acid (**2**) with methyl chloroformate, using an unusual procedure involving solid Na_2SO_4 in dioxane. These acidic conditions are therefore a good alternative to Schotten−Baumann-type procedures, which can give rise to side reactions of carboxylate with chloroformate, as some-

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/eurjoc or from the author.

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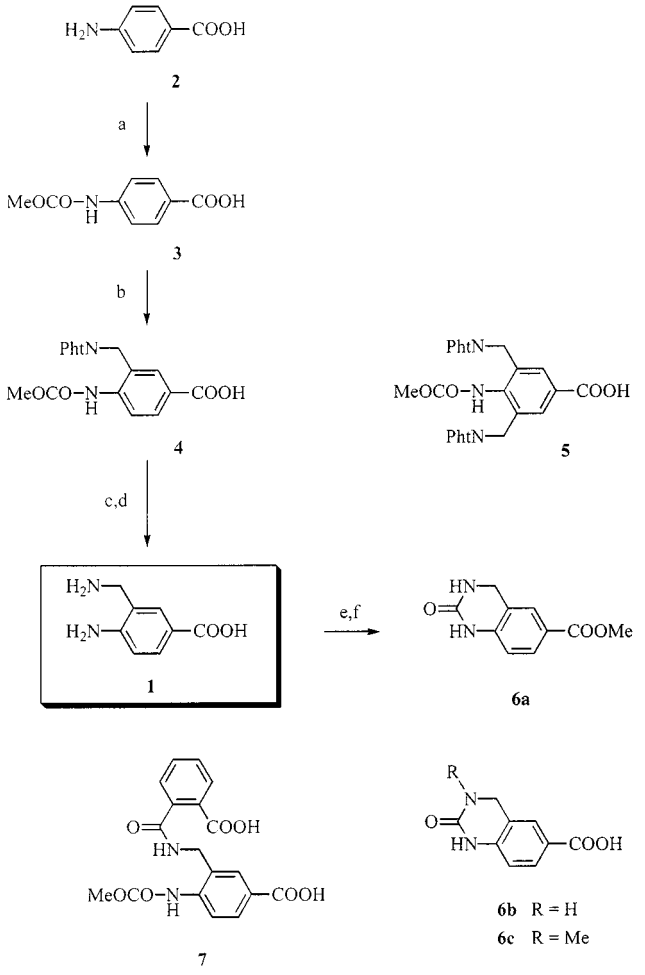


Scheme 1. Synthesis of AmAbz, **1**: (a) MeOCOCl, Na_2SO_4, dioxane, 70 °C, 97%; (b) *N*-hydroxymethylphthalimide, 96% H_2SO_4/H_2O (9:1), 50 °C, 73%; (c) 5 N NaOH, reflux, 48 h; (d) HCO_2H, purification on cation exchange resin, 89%; (e) $SOCl_2$, MeOH; (f) *N*,*N*′-carbonyldiimidazole, DIEA, dioxane

times observed during protection of amino acids with urethane-type blocking groups.[14] Such side reactions might be favoured by the low nucleophilicity of the arylamine in amino acid **2**. Under the moderately acidic conditions selected, the reactivity of the arylamino group (pK_A = 2.38 in water[15]) was preserved, while that of the carboxyl group was suppressed, with no need to start from the methyl ester[16] or to convert the carboxyl group into a trimethylsilyl ester[17] as previously described in some syntheses of 4-aminobenzoic acid carbamates.

Amidomethylation of aromatic compound **3** with hydroxymethylphthalimide was carried out in 96% H_2SO_4/H_2O (9:1) at 50 °C and took place exclusively at the position *ortho* to the carbamate group, as unambiguously confirmed by subsequent preparation of the urea **6a** (vide infra). The only side product (**5** ≈ 8%) was identified as an *ortho,ortho*′-disubstituted compound with two aromatic hydrogen atoms in a symmetrical structure, as attested by ^{1}H NMR analysis on the isolated substance. This side product was efficiently removed by recrystallization, and the desired product **4** was obtained in 73% yield.

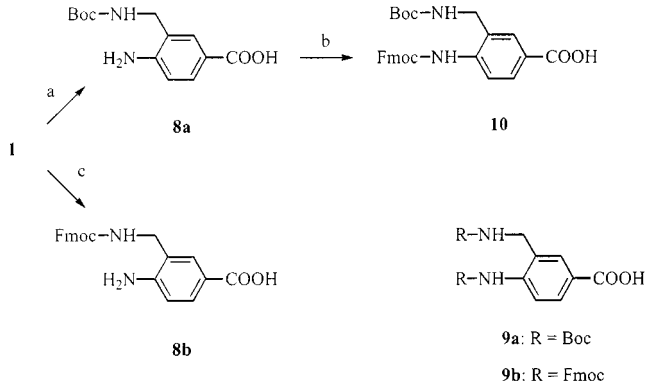
Deblocking of both amino groups of compound **4** by quantitative hydrolysis under strongly alkaline conditions afforded AmAbz (**1**).[18] It was isolated either by means of a tedious separation on sulfonic acid ion exchange resin (89% yield) or, alternatively, obtained as its phthalic acid salt, which readily precipitated upon acidification of the reaction medium (67% yield). Both forms could be used efficiently for further preparation of protected derivatives. It should be noted that hydrolysis of **4** in weakly alkaline carbonate buffers gave the substituted phthalamic acid **7** in high yield, confirming that the alkaline hydrolysis occurs by ring-opening of the phthalimide. Urea **6b** was also isolated in significant yield after incomplete hydrolysis with NaOH, indicating that hydrolysis occurs, at least in part, through an intramolecular pathway, which is consistent with the ease of cyclization of 2-aminobenzylamine carbamates.[19] The relative positions of substituents in AmAbz (**1**) were confirmed by its ability to undergo the intramolecular reaction to give the cyclic urea **6a**[20] (Scheme 1). This urea was identified by comparison of its NMR spectrum with the published data for the 3-methyl derivative **6c**.[21]

Protection of AmAbz

The difference in nucleophilicities between the aryl amine and the benzyl amine of AmAbz (pK_A values of 2.38[15] and 9.33[22] for 4-aminobenzoic acid and benzylamine, respectively) was used in the protection strategy to discriminate between the two amino groups. In alkaline dioxane/water, Boc_2O or Fmoc−OSu reacted with high regioselectivity to give the monoprotected benzylamines **8a** or **8b**, respectively (Scheme 2). The amounts of bis-protected side products **9a** and **b** were lower than 1% (by HPLC analysis of the reaction medium or ^{1}H NMR analysis of the crude product), provided that **1** was maintained in slight excess. We noticed, however, that significant amounts (≈ 4%) of the bis-Fmoc side product **9b** were obtained when Fmoc−Cl was used instead of Fmoc−OSu under identical conditions (data not given). In view of this reactivity, protection of the arylamino group to obtain **10** then proved feasible, by treatment of **8a** with Fmoc−Cl in dioxane at 70 °C in the presence of Na_2SO_4. In contrast, however, introduction of the Boc protecting group onto AmAbz(Fmoc) **8b** proved to be much more difficult. It remains the case though that coupling of AmAbz derivatives was possible without any protection of the arylamino group (vide infra).

Peptide Bond Formation Involving AmAbz Derivatives

We first studied the acylation of an amino acid methyl ester by AmAbz(Fmoc) **8b**. When **8b** (0.1 M in DMF) was allowed to react with BOP reagent (1 equiv.) in the presence of HOBt (1 equiv.) and DIEA (2 equiv.), HPLC monitoring detected the formation of only one species (Supporting Information): presumably the active ester AmAbz(Fmoc)−OBt which suffered no degradation even after 2 h in DMF at room temperature. This species was capable of subsequently acylating Ala−OMe to give the desired dipeptide ester AmAbz(Fmoc)−Ala−OMe **11** in a

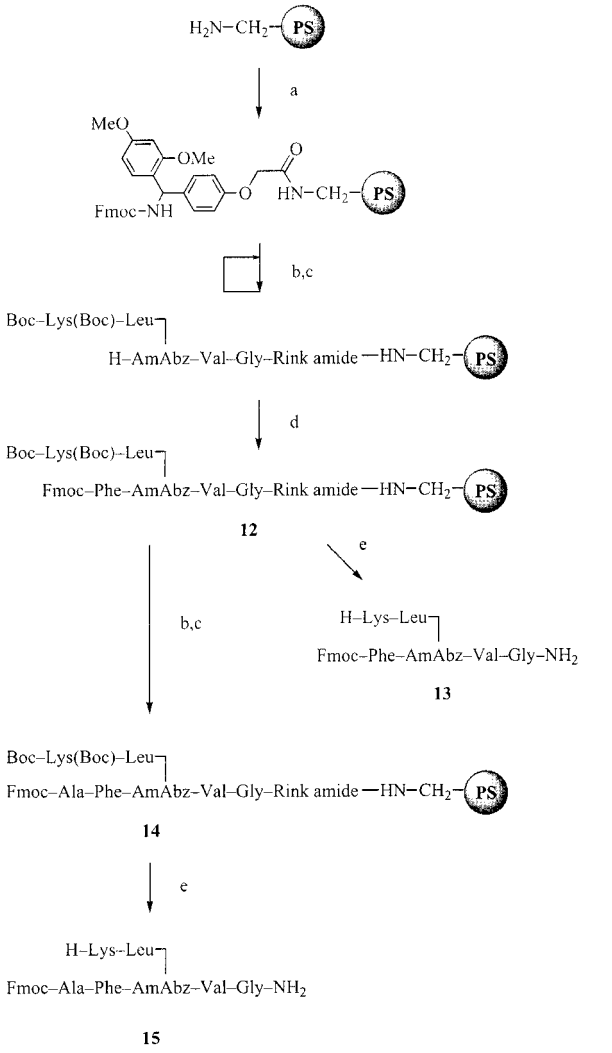


Scheme 2. Protection of AmAbz: (a) Boc_2O, NaOH, dioxane/H_2O, 0 °C, 86%; (b) Fmoc−Cl, Na_2SO_4, dioxane, 70 °C, 69%; (c) Fmoc−OSu, Na_2CO_3, NaOH, dioxane/H_2O, 0 °C, 91%

clean reaction, as shown by HPLC analysis of the reaction medium. These results demonstrated that protection of the arylamino group is not necessary for acylation with AmAbz derivatives, which is consistent with results obtained in the related 3,5-diaminobenzoic acid series.[6] They also suggested the possibility of continuing peptide elongation from the benzylamino group without protection of the arylamino group.

We took advantage of these results for the solid-phase synthesis of the AmAbz-containing branched pseudopeptides **13** and **15** (Scheme 3), starting from aminomethylpolystyrene resin derivatized with the Rink amide linker.[23] Coupling reactions were carried out using the BOP/HOBt/DIEA methodology, with the exception of that of Fmoc−Phe. As anticipated, Leu and Lys residues could be introduced without protecting the arylamino group of AmAbz. The arylamino group was then acylated with Fmoc−Phe using DIC activation in CH_2Cl_2 in the absence of base:[24] conditions under which any racemization of activated amino acid derivatives was expected to be prevented. This method was therefore preferred to the use of PyBrop[25] in DMF, recently recommended for acylation of the arylamino group of 3,5-diaminobenzoic acid residue,[26] but which requires the presence of a base. DIC activation was selected on the basis of preliminary experiments[27] that showed that acylation of the arylamino group of AmAbz was remarkably fast and complete when a base-free, carbodiimide-mediated coupling reaction in CH_2Cl_2 was used,[28] contrasting sharply with the inadequacy mentioned above of BOP activation in DMF. This efficiency is in agreement with literature data reporting that the activation of carboxylic acids with carbodiimides is highly solvent-dependent[29−31] and remarkably fast in dichloromethane;[31,32] addition of bases is also generally unfavourable.[32,33]

The high purity of crude pseudopeptides **13** and **15** as assessed by HPLC and MALDI-MS analyses (Supporting Information) demonstrates the efficiency of this synthetic method. In particular, the MALDI mass spectra displayed the expected masses at *m/z* = 932.5 and 1003.3, respectively, with no trace of the side products that would have been formed by premature acylation of the arylamine:



Scheme 3. Solid-phase synthesis of peptides **13** and **15** from aminomethyl polystyrene resin: (a) Fmoc-Rink amide linker (1.5 equiv.), BOP (1.5 equiv.), DIEA (2.25 equiv.), DMF, 90 min, then Ac_2O (10 equiv.), DIEA (3.0 equiv.), DMF, 15 min; (b) piperidine/DMF (1:4) 1 × 1 min + 3 × 3 min; (c) protected amino acid (Fmoc−Gly−OH, Fmoc−Val−OH, H−AmAbz(Fmoc)−OH, Fmoc−Leu−OH, Boc−Lys(Boc)−OH or Fmoc−Ala−OH) (3.3 equiv.), HOBt (3.0 equiv.), BOP (3.0 equiv.), DIEA (4.5 equiv.), DMF, 60 min; (d) Fmoc−Phe (3.3 equiv.), DIC (3.0 equiv.), CH_2Cl_2, 2 × 3 h (with DMF addition for the second coupling); (e) TFA/H_2O/triisopropylsilane (95:2.5:2.5), 3 h

H−Lys−AmAbz(H−Lys−Leu)−Val−Gly−NH_2 (*m/z* = 691.5) or H−Lys−Leu−AmAbz(H−Lys−Leu)−Val−Gly−NH_2 (*m/z* = 804.5). Additionally, acylation of the arylamine with Fmoc−Phe was shown to be quantitative, since no pentapeptide H−AmAbz(H−Lys−Leu)−Val−Gly−NH_2 (*m/z* = 563.4) was detected.

Conclusion

We have shown experimentally that AmAbz is an easily accessible new dipeptide mimic that can be converted into building blocks for branched peptide synthesis using mild coupling methods. Further applications of this compound as a scaffold in combinatorial chemistry are under study. In

addition, our background in the design of safety-catch linkers for solid-phase synthesis[34,35] had led us to anticipate that derivatives of AmAbz might have useful applications in this field, thanks to their potential to be converted into highly reactive cyclic *N*-acylureas.[35] We have therefore undertaken studies aimed at evaluating the reactivity of such ureas with various nucleophiles for further applications in convergent synthesis of peptides or in peptide ligation.

Experimental Section

General Remarks: TLC: SDS precoated plates (0.2 mm), silica gel 60, F254; detection: UV and/or spraying with a solution of ninhydrin (1 g) in 95% EtOH (100 mL) and heating. − Melting points: Electrothermal IA9200. − Elemental analyses were performed by the "Service Central d'Analyse", CNRS, Vernaison (France). − ^{1}H NMR: Bruker Avance DPX 200 (200 MHz). [D$_6$]DMSO as solvent δ_H = 2.50. − MS: Bruker Biflex III (MALDI-TOF); Jeol SX 102 (FAB$^+$). − Analytical HPLC: Buffer A 0.1% aqueous TFA, B CH_3CN (0.1% TFA); System A: Beckman System Gold Chromatograph (Programmable Solvent Module 126, Diode Array Detector Module 168, column Kromasil C8 5µm 4.6 × 150 mm, linear gradient 50 to 100% buffer B over 10 min, flow 1.5 mL/min); System B: Merck chromatograph (655A−11 pump, 655A UV/Vis detector (λ = 214 nm), L-5000 LC Controller, D-2000 Chromato Integrator, column LiChrospher 100 RP-18, linear gradient 20 to 60% buffer B over 30 min, flow 2 mL/min).

4-(Methoxycarbonylamino)benzoic Acid (3): A mixture of 4-aminobenzoic acid **2** (13.7 g, 100 mmol) and Na_2SO_4 (28.4 g, 200 mmol, added to combine with the liberated HCl) in dioxane (100 mL) was heated to 70 °C with vigorous stirring (heating ensured complete dissolution of **2**). A solution of MeOCOCl (7.8 mL, 100 mmol) in dioxane (20 mL) was slowly added over 2 h. The mixture was further stirred for 15 min at 70 °C. After cooling to room temperature, water (50 mL) was added and the mixture was concentrated under reduced pressure. A solid precipitated upon addition of water (100 mL). It was collected by filtration, washed repeatedly with water and dried in vacuo to give crude **3** (18.9 g, 97%), which was used in the next step without further purification. White crystals were obtained by recrystallization from EtOH/water (1:4). − M.p. 204.5 °C (ref.[36] 207 °C,[16] 195−196 °C for **3** · 0.25 H_2O). − TLC: R_f = 0.65 (CH_2Cl_2/MeOH/AcOH, 10:1:0.15). − For ^{1}H NMR spectrum see ref.[16]

4-Methoxycarbonylamino-3-(phthalimidomethyl)benzoic Acid (4): A mixture of **3** (9.76 g, 50.0 mmol), water (5 mL) and concentrated H_2SO_4 (45 mL) was heated to 50 °C with stirring. Solid *N*-hydroxymethylphthalimide (8.86 g, 50.0 mmol) was added in portions over 30 min. The solution was stirred for 2 h at 50 °C, cooled to room temperature, and slowly poured into EtOH/water (1:3, 400 mL) with vigorous stirring. The product was collected by filtration, repeatedly washed with water to neutrality and dried in vacuo to give a solid (16.3 g) containing **4** (85% molar ratio by NMR), residual carbamate **3** (7%) and bis-adduct **5** (8%). The product was recrystallized from DMF/AcOH (1:5), washed with AcOH, EtOH, and Et_2O and dried in vacuo to give white crystals (13.5 g) containing residual AcOH (4.6% by weight), corresponding to a 73% yield. This product was used in the next step. Recrystallization from damp MeOH gave a pure sample of the hemihydrate **4** · $^1/_2$ H_2O, m.p. 263 °C (dec.). − TLC: R_f = 0.39 (toluene/dioxane/AcOH, 95:25:4). − ^{1}H NMR ([D$_6$]DMSO): δ = 3.71 [s, 3 H, OCH_3], 4.81 [s, 2 H, CH_2], 7.62 [d, $J_{5,6}$ = 8.4 Hz, 1 H, 5-H], 7.68 [d, $J_{2,6}$ = 1.9 Hz, 1 H, 2-H], 7.83 [dd, $J_{5,6}$ = 8.4 Hz, $J_{2,6}$ = 1.9 Hz, 1 H, 6-H], 7.84−7.93 [m, 4 H, aromatic H], 9.38 [s, 1 H, NH], 12.88 [s, 1 H, COOH]. − MS (FAB): *m/z* 355 [M + H$^+$]. − $C_{18}H_{14}N_2O_6 \cdot 0.5\ H_2O$ (363.32): calcd. C 59.52, H 4.16, N 7.71; found C 59.82, H 4.22, N 7.79.

Isolation of 5: The filtrate remaining after recrystallization of **4** from DMF/AcOH was concentrated under reduced pressure. A solid precipitated on addition of water. After filtration, the solid was recrystallized from DMF/AcOH (1:1) to give compound **5** (1.05 g, 4%) as a white solid, m.p. 283 °C (dec.). − TLC: R_f = 0.24 (toluene/dioxane/AcOH, 95:25:4). − ^{1}H NMR ([D$_6$]DMSO): δ = 3.62 [s, 3 H, OCH_3], 4.79 [s, 4 H, CH_2], 7.66 [s, 2 H, aromatic H], 7.86−7.94 [m, 8 H, aromatic H], 9.31 [s, 1 H, NH], 13.10 [s, 1 H, COOH]. − MS (FAB): *m/z* 514 [M + H$^+$].

4-Amino-3-(aminomethyl)benzoic Acid (1): A mixture of compound **4** (21.3 g, 60 mmol), NaOH (16.8 g, 420 mmol) and water (60 mL) in a Teflon flask was stirred and heated at reflux. The reaction was complete after 48 h, as shown by TLC analysis (butyl alcohol/AcOH/water, 2:1:1) indicating the presence of the hydrolysis products only: **1** (R_f = 0.70) and phthalic acid (R_f = 0.75). The solution was cooled to room temperature and then partially neutralized with AcOH (10.7 mL), which caused precipitation. The filtrate and the solid (which dissolved in a large volume of water) were introduced onto a column filled with Dowex 50 (H$^+$-form) ion exchange resin. The resin was washed with water to remove phthalic acid, and the product was eluted with 0.2 M ammonia. Removal of the solvent under reduced pressure and drying in vacuo gave amino acid **1** (9.4 g, 89%) as a hemihydrate, m.p. > 250 °C (dec.). − TLC: R_f = 0.70 (butyl alcohol/AcOH/water, 2:1:1). − ^{1}H NMR (D_2O): δ = 3.99 [s, 2 H, CH_2], 6.69−6.74 [m, 1 H, aromatic H], 7.54−7.60 [m, 2 H, aromatic H]. − MS (FAB): *m/z* (%) 167 (100) [M + H$^+$], 150 (85) [M + H$^+$ − NH_3]. − $C_8H_{10}N_2O_2 \cdot 0.5\ H_2O$ (175.18): calcd. C 54.85, H 6.33, N 15.99; found C 54.66, H 6.25, N 15.81.

Phthalic Acid Salt of 1: To the alkaline solution obtained after a similar hydrolysis of compound **4** (7.08 g, 20 mmol) with NaOH (4.80 g, 120 mmol) in 25 mL of water was added H_2SO_4 (2.5 N, 50 mL). The precipitate was filtered, washed with water, EtOH, and Et_2O, and dried in vacuo to give **1** as a phthalic acid salt (4.48 g, 67%), m.p. > 300 °C (dec.). − TLC: R_f = 0.70 and R_f = 0.75 (butyl alcohol/AcOH/water, 2:1:1). − ^{1}H NMR ([D$_6$]DMSO): δ = 3.94 [s, 2 H, CH_2], 6.12 [broad s, 2 H, NH_2], 6.69 [d, $J_{5,6}$ = 8.5 Hz, 1 H, 5-H], 7.45−7.54 [m, 2 H, aromatic H], 7.65 [dd, $J_{5,6}$ = 8.5 Hz, $J_{2,6}$ = 2.0 Hz, 1 H, 6-H], 7.79 [d, $J_{2,6}$ = 2.0 Hz, 1 H, 2-H], 8.11−8.19 [m, 2 H, aromatic H].

Methyl 1,2,3,4-Tetrahydro-2-oxo-6-quinazolinecarboxylate (6a): $SOCl_2$ (0.88 mL, 12.1 mmol) was added carefully to MeOH (50 mL) at 0 °C with vigorous stirring. Amino acid **1** (1.00 g, 5.7 mmol) was added to the resulting solution and the mixture was heated at reflux for 5 h, then concentrated under reduced pressure. Recrystallization of the residue from MeOH gave the methyl ester dihydrochloride (1.49 g), m.p. 209 °C (dec.). − ^{1}H NMR (D_2O): δ = 3.72 [s, 3 H, OCH_3], 4.05 [s, 2 H, CH_2], 6.90 [d, $J_{5,6}$ = 8.4 Hz, 1 H, 5-H], 7.74 [dd, $J_{5,6}$ = 8.0 Hz, $J_{2,6}$ = 2.0 Hz, 1 H, 6-H], 7.80 [d, $J_{2,6}$ = 2.0 Hz, 1 H, 2-H]. This salt (0.200 g, 0.79 mmol) was allowed to react in dioxane (10 mL) with *N*,*N*′-carbonyldiimidazole (0.253 g, 1.56 mmol) and DIEA (0.27 mL, 1.56 mmol) for 2.5 h at 80 °C. The solution was cooled to room temperature and poured into water. The precipitate was filtered, washed with water and dried in vacuo. Recrystallization from isopropyl alcohol gave **6a** as a white solid (0.080 g), m.p. 259 °C. − TLC: R_f = 0.35 (CH_2Cl_2/

MeOH, 10:1). − ^{1}H NMR ([D$_6$]DMSO): δ = 3.78 [s, 3 H, OCH$_3$], 4.36 [s, 2 H, CH$_2$], 6.82 [d, $J_{5,6}$ = 8.9 Hz, 1 H, 5-H], 7.02 [broad s, 1 H, CH$_2$−N*H*], 7.70−7.74 [m, 2 H, 2,6-H], 9.45 [broad s, 1 H, NH]. − MS (FAB): *m*/*z* 207 [M + H$^+$]. − $C_{10}H_{10}N_2O_3$ (206.20): calcd. C 58.25, H 4.89, N 13.59; found C 58.26, H 4.49, N 13.41.

1,2,3,4-Tetrahydro-2-oxo-6-quinazolinecarboxylic Acid (6b): A solution of **4** (0.354 g, 1.00 mmol) in NaOH (1 N, 3.5 mL) was heated at 100 °C for 24 h. The solid formed upon acidification to pH 1 with concentrated HCl was collected by filtration, washed with water and dried in vacuo to give 32 mg (16%) of compound **6b**, m.p. > 275 °C (dec.). − TLC: R_f = 0.80 (butyl alcohol/AcOH/water, 2:1:1). − ^{1}H NMR ([D$_6$]DMSO): δ = 4.35 [s, 2 H, CH$_2$], 6.80 [d, $J_{5,6}$ = 8.0 Hz, 1 H, 5-H], 6.98 [s, 1 H, CH$_2$−N*H*], 7.67−7.72 [m, 2 H, 2,6-H], 9.38 [s, 1 H, NH], 12.56 [broad s, 1 H, COOH].

3-(2-Carboxybenzoylaminomethyl)-4-(methoxycarbonylamino)-benzoic Acid (7): A mixture of **4** (0.177 g, 0.50 mmol), Na$_2$CO$_3$ (0.212 g, 2.00 mmol) and water (2 mL) was heated at 60 °C for 3 h. The medium was acidified to pH 1 with concentrated HCl, the solid was collected by filtration, washed with water and dried to give 0.17 g (91%) of compound **7**. M.p. > 220 °C (dec.). − ^{1}H NMR ([D$_6$]DMSO): δ = 3.70 [s, 3 H, OCH$_3$], 4.42 [d, J = 6.1 Hz, 2 H, CH$_2$], 7.39−7.94 [m, 7 H, aromatic H], 9.11 [t, J = 6.1 Hz, 1 H, CH$_2$N*H*], 9.75 [s, 1 H, NH], 12.9 [broad s, 2 H, COOH]. − MS (FAB): *m*/*z* 373 [M + H$^+$].

AmAbz(Boc) (8a): A solution of Boc$_2$O (1.244 g, 5.70 mmol) in dioxane was prepared (3 mL). A suspension of **1** (hemihydrate, 1.051 g, 6.00 mmol) in water (6 mL) and dioxane (12 mL), stirred and cooled to 0 °C, was subjected five times to the following treatment: addition of NaOH (2.5 N, 0.93 mL) immediately followed by addition of a 0.6 mL aliquot of the Boc$_2$O solution, with a 10 min interval between each Boc$_2$O addition. The mixture was further allowed to react for 1 h at room temperature, then diluted with water and washed with *t*BuOMe. The aqueous layer was acidified with formic acid (0.6 mL, 2.5 mmol) and extracted with EtOAc. The organic layer was washed with water and with brine, and then was dried (Na$_2$SO$_4$) and concentrated to ca. 5 mL. Addition of pentane gave a solid, which was collected by filtration, washed with pentane and dried in vacuo to give 1.315 g (86%) of pure **8a**. M.p. > 190 °C (dec.). − TLC: R_f = 0.62 (CH$_2$Cl$_2$/MeOH/AcOH, 10:1:0.15). − ^{1}H NMR ([D$_6$]DMSO): δ = 1.39 [s, 9 H, C(CH$_3$)$_3$], 3.95 [d, J = 6.1 Hz, 2 H, CH$_2$], 5.79 [broad s, 2 H, NH$_2$], 6.59 [d, J = 8.3 Hz, 1 H, aromatic H], 7.31 [t, J = 6.1 Hz, 1 H, CH$_2$N*H*], 7.51−7.57 [m, 2 H, aromatic H], 12.0 [broad s, 1 H, COOH]. − MS (FAB): *m*/*z* 267 [M + H$^+$]. − $C_{13}H_{18}N_2O_4$ (266.29): calcd. C 58.63, H 6.81, N 10.52; found C 58.36, H 6.78, N 10.23.

Alternatively, a suspension of the phthalic acid salt of **1** (1.66 g, 5.0 mmol) in dioxane (10 mL), NaOH (2.5 N, 4 mL), and water (1 mL) was treated similarly to give **8a** (1.024 g, 81%).

AmAbz(Fmoc) (8b): Amino acid **1** (2.00 mmol) was allowed to react with Fmoc−OSu (1.90 mmol) as follows. A suspension of **1** (hemihydrate, 0.350 g), Na$_2$CO$_3$ (0.265 g, 2.50 mmol) in dioxane (10 mL), and water (5 mL) was vigorously stirred, then cooled to 0 °C. Addition of NaOH (2.5 N, 0.16 mL), immediately followed by addition of solid Fmoc−OSu (0.128 g), was performed five times, with a 10 min interval between each Fmoc−OSu addition. The mixture was further allowed to react for 1 h at room temperature, then diluted with water and washed with *t*BuOMe (40 mL); the organic layer was extracted with 5% (aq.) NaHCO$_3$ (ca. 10 mL). The combined aqueous phases were acidified to pH 1 with concentrated H$_2$SO$_4$, then extracted with EtOAc. The organic layer was washed with 5% KHSO$_4$, water and brine, then dried (Na$_2$SO$_4$) and concentrated to ca. 5 mL. The solid which precipitated was collected by filtration, washed with a small volume of cold EtOAc and then with pentane. It was dried to give **8b** as a white solid (0.514 g, 66%), m.p. > 200 °C (dec.). An additional fraction of **8b** (0.184 g, 25%, HPLC purity ca. 95%) was obtained from the filtrate after removal of the solvent under reduced pressure, dissolving of the residue in EtOH and precipitation by addition of water. − TLC: R_f = 0.4 (EtOAc). − HPLC: t_R = 4.1 min (system A). − ^{1}H NMR ([D$_6$]DMSO): δ = 4.02 [d, J = 6.0 Hz, 2 H, CH$_2$], 4.2−4.35 [m, 3 H, CH−CH$_2$], 5.79 [s, 2 H, NH$_2$], 6.62 [d, J = 8.4 Hz, 1 H, aromatic H], 7.28−7.90 [m, 11 H, aromatic H and CH$_2$N*H*], 12.03 [s, 1 H, COOH]. − MS (FAB): *m*/*z* 389 [M + H$^+$]. − $C_{23}H_{20}N_2O_4$ (388.42): calcd. C 71.12, H 5.19, N 7.21; found C 71.20, H 5.29, N 7.18.

Fmoc−AmAbz(Boc) (10): Na$_2$SO$_4$ (0.763 g, 5.37 mmol) was added to a solution of **8a** (0.715 g, 2.68 mmol) in dioxane (5 mL). The mixture was vigorously stirred and heated to 70 °C. Next, a solution of Fmoc−Cl (0.695 g, 2.68 mmol) in dioxane (0.7 mL) was added dropwise over 60 min. The mixture was cooled to room temperature and allowed to react further for 3 h. Complete precipitation was accomplished on addition of water with vigorous stirring. The precipitate was collected by filtration, washed and triturated with water, then dried in vacuo. The solid was dissolved in MeOH, and the solution was concentrated under reduced pressure. A precipitate was obtained, collected by filtration and dried to give **10** (0.907 g, 69%). − M.p. > 200 °C (dec.). − TLC: R_f = 0.67 (CH$_2$Cl$_2$/MeOH/AcOH, 10:1:0.15). − ^{1}H NMR ([D$_6$]DMSO): δ = 1.42 [s, 9H, C(CH$_3$)$_3$], 4.15 [d, J = 6.0 Hz, 2 H, CH$_2$], 4.31 [t, J = 6.8 Hz, 1 H, C*H*−CH$_2$], 4.44 [d, J = 6.8 Hz, 2 H, CH−C*H*$_2$], 7.29−7.47 [m, 4 H, aromatic H], 7.5−7.7 [m, 2 H, aromatic H and CH$_2$N*H*], 7.75−7.93 [m, 6 H, aromatic H], 9.65 [s, 1 H, NH], 12.81 [s, 1 H, COOH]. − MS (FAB): *m*/*z* (%) 489 (3) [M + H$^+$], 433 (4) [M + H$^+$ − *t*Bu], 389 (28) [M + H$^+$ − Boc], 179 (100) [dibenzofulvenylium cation]. − $C_{28}H_{28}N_2O_6 \cdot 0.25\ CH_3OH$ (496.54): calcd. C 68.33, H 5.89, N 5.64; found C 67.86, H 5.84, N 5.63.

Reaction of 8b with BOP Reagent and Formation of AmAbz(Fmoc)−Ala−OMe (11): A solution of BOP (88 mg, 0.20 mmol) in DMF (1.8 mL) was poured into a test tube containing AmAbz(Fmoc) **8b** (78 mg, 0.20 mmol) and HOBt · H$_2$O (31 mg, 0.20 mmol). After addition of DIEA (70 μL, 0.40 mmol), the mixture was allowed to react at room temperature. The reaction was monitored by HPLC analysis (System A) of samples (50 μL) withdrawn from the reaction medium and diluted with 2.5 mL CH$_3$CN/water (4:1). Compound **8b** (t_R = 4.1 min) was quickly converted (> 95% at 2 min, > 99% at 30 min) into the activated ester (t_R = 6.8 min); after 2 h, no further change was observed, and Ala−OMe · HCl (28 mg, 0.20 mmol) with DIEA (35 μL, 0.20 mmol) were added to the mixture, resulting in the formation of one product (t_R = 4.4 min, 85% at 60 min); the reaction was then forced to completion by addition of further Ala−OMe · HCl (5 mg) and standing overnight at room temperature. The reaction medium was diluted with EtOAc (20 mL), washed repeatedly with water, 5% Na$_2$CO$_3$, 5% KHSO$_4$, water and brine, then dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give **11**. − HPLC: t_R = 4.4 min (system A). − ^{1}H NMR ([D$_6$]DMSO): δ = 1.37 [d, J = 7.3 Hz, 3 H, CH−C*H*$_3$], 3.62 [s, 3 H, O−CH$_3$], 4.01 [d, J = 5.8 Hz, 2 H, CH$_2$], 4.19−4.50 [m, 4 H, αCH and CH−CH$_2$], 5.60 [broad s, 2 H, NH$_2$], 6.64 [d, $J_{5,6}$ = 8.3 Hz, 1 H, 5-H], 7.28−7.89 [m, 11 H, aromatic H and N*H*−CH$_2$], 8.33 [d, J = 7.0 Hz, 1 H, N*H*−CH]. − MS (MALDI): *m*/*z* (%) 474 (14) [M + H$^+$], 496 (100) [M + Na$^+$], 512 (100) [M + K$^+$].

Resin 12: Solid-phase syntheses were carried out using a glassware manual apparatus involving a reaction vessel equipped with a fritted disc, a solvent inlet and a device to apply low nitrogen pressure used to fill the vessel with DMF or CH_2Cl_2 and to remove liquid waste. Continuous "stirring" was performed during all the washing and coupling steps, either by gently rocking the vessel on a shaker or by bubbling nitrogen through the fritted plate. The efficiency of the coupling reactions was monitored using the ninhydrin test. Commercial aminomethylpolystyrene resin (1.00 g, 0.56 mmol/g, NovaBiochem) was introduced into the reaction vessel and subjected to the following washes: DMF (2 × 1 min + 1 × 30 min); CH_2Cl_2 (3 × 1 min); TFA/CH_2Cl_2 (1:1) (1 × 10 min); DIEA/CH_2Cl_2 (1:20) (2 × 10 min); CH_2Cl_2 (4 × 1 min); DMF (4 × 1 min). Then, Fmoc−Rink amide linker (0.45 g, 0.84 mmol, NovaBiochem) was allowed to react with the resin for 90 min, using BOP (0.37 g, 0.84 mmol), DIEA (0.22 mL, 1.26 mmol), and DMF. Unreacted amino groups were acetylated by reaction with Ac_2O (0.53 mL, 5.6 mmol) and DIEA (0.29 mL, 1.68 mmol) in DMF for 15 min. Next, amino acids were incorporated as follows: (i) Fmoc removal: piperidine/DMF (1:4) (1 × 1 min + 3 × 3 min); DMF washes (4 × 1 min); (ii) coupling reaction: the protected amino acid (1.85 mmol, 3.3 equiv.) and HOBt (0.26 g, 1.68 mmol) were dissolved in the minimum possible volume of DMF and then introduced into the reaction vessel, DIEA (0.44 mL, 2.52 mmol) and solid BOP (0.743 g, 1.68 mmol) were added and stirring was continued for 60 min; DMF washes (4 × 1 min). An acetylation step was inserted after the Fmoc−Gly coupling reaction because of a slightly positive ninhydrin test. A different procedure was used for the Fmoc−Phe coupling reaction: DMF washes (4 × 1 min); CH_2Cl_2 washes (4 × 1 min); Fmoc−Phe (0.716 g, 1.85 mmol) was dissolved in CH_2Cl_2 and then introduced into the reaction vessel; coupling was initiated by addition of DIC (0.263 mL, 1.68 mmol). CH_2Cl_2 was added 5 min later because gelation of the medium was observed, making it necessary to resuspend the resin. The suspension was allowed to react for 3 h. Since no test for detecting unchanged arylamino groups was available (the ninhydrin test is inefficient), a second coupling was performed using a similar procedure except that DMF was added after 5 min and CH_2Cl_2 was evaporated off by nitrogen bubbling during the reaction. Finally, the resin was washed repeatedly with DMF, CH_2Cl_2, MeOH, 100% EtOH and Et_2O, and dried, first by flushing nitrogen through the vessel (10 min) and then under vacuum, yielding resin **12** (1.475 g, theoretically 1.79 g based on the loading of the starting support).

Peptide 13: This was released into solution by shaking a suspension of resin **12** (50 mg) in TFA/water/triisopropylsilane (95:2.5:2.5, 2 mL) for 3 h. The suspension was filtered and the resin was washed with TFA (2 × 1 mL). The filtrates were concentrated, then diluted with Et_2O (100 mL) and extracted twice with water (25 mL then 10 mL). The combined aqueous phases were washed with Et_2O (3 × 50 mL), then concentrated and freeze-dried to give **13** (7.6 mg). − HPLC: t_R = 18.7 min (system B). − MS (MALDI): *m/z* 932.5 (100) [M + H^+], 954.5 (60) [M + Na^+], 970.4 (31) [M + K^+].

Peptide 15: The Fmoc group was removed from resin **12** (200 mg) and then Fmoc−Ala (65 mg, 0.21 mmol) was coupled with the resulting resin using the BOP/HOBt/DIEA method described above. The resin was washed and dried to give resin **14** (209 mg). A suspension of **14** in TFA/water/triisopropylsilane (95:2.5:2.5, 8 mL) was shaken for 3 h, then treated as described above to yield crude peptide **15** (19.7 mg). − HPLC: t_R = 18.6 min (system B). − MS (MALDI): *m/z* (%) 1003.3 (100) [M + H^+], 1025.3 (25) [M + Na^+], 1041.2 (5) [M + K^+].

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