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Direct solid-phase synthesis of quinoxaline-containing peptides

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Dedicated to Professor Ignacy Z. Siemion on the occasion of 50 years of his scientific activity

Abstract—The solid-phase synthesis of a series of model dipeptides containing various 3-(quinoxalin-6-yl)alanine analogues is described. The method involves formation of a quinoxaline heterocycle by condensation between an α-dicarbonyl compound and a β-(3,4-diaminophenyl)alanine residue, immobilized on a solid support.

Although peptides and proteins mediate numerous biological processes, their use as therapeutic agents is often accompanied by several drawbacks, including poor bioavailability, low biostability, and limited receptor subtype selectivity. Therefore, much effort has been expended on the design and synthesis of selective, high-affinity ligands by replacing portions of peptides with nonpeptide structures and/or certain unnatural amino acids. The synthesis of unnatural amino acids has attracted a great deal of attention[1] due to the diverse range of biological and toxicological properties exhibited by these compounds. Nonproteinogenic (especially heterocyclic) amino acids incorporated into polypeptides have been used to probe enzyme structure and function or to develop new chemical libraries for DNA binding.[2]

There are many examples of biologically active quinoxalines. They show very interesting pharmacological properties (antibacterial, antiviral, anticancer, antifungal, anthelmintic, and insecticidal) and will be of interest in medicinal chemistry for some time to come.[3] Some quinoxaline analogues such as 2,3-bis(2-pyridyl)quinoxaline (DPQ),[4] dipyridophenazine (DPPZ), and 2,3-bis(2-furanyl)quinoxaline (DFQ), complexed with transition metals are of current interest in view of their binding to DNA. They are known as 'chemical nucleases' that efficiently 'nick' DNA. DFQ derivatives show antispasmodic activity.[5] Several synthetic strategies have been developed for the preparation of substituted quinoxalines.[6] The most common method relies on the condensation of an aryl 1,2-diamine with an α-dicarbonyl compound. Previously, α-amino acids with quinoxaline side chains have been synthesized in solution only.[7] In the last decade, the emergence of combinatorial chemistry has stimulated intensive efforts in the application of common reactions to solid-phase synthesis. The strategy offers the opportunity of rapidly synthesizing molecules without the need for tedious and time-consuming purification.[8] Although many heterocycles can be prepared efficiently through cyclization on solid supports,[9] solid-phase heterocyclic chemistry of peptides is relatively unexplored.

In a previous letter, Wu and Ede reported the first solid-phase synthesis of quinoxalines, in which *o*-phenylenediamine bound to SynPhase™ Lanterns was condensed with α-bromoketones in DMF at 60 °C to give quinoxalines.[10] However, we did not use these precursors for peptide synthesis, because their high reactivity creates the possibility of side reactions in syntheses of more complex peptides. Other disadvantages of α-bromoketones are their toxicity and lachrymatory properties. Moreover, most commercially available α-bromoketones produce a mixture of quinoxaline isomers,[10] which requires a tedious separation. Herein, we report the incorporation of quinoxaline moieties into a model dipeptide by reaction between the β-(3,4-diaminophenyl)alanine residue of a peptide immobilized on Wang resin and symmetrical α-dicarbonyl compounds, which are free from the disadvantages of the bromoketones (Scheme 1). In order to prepare peptidyl resin

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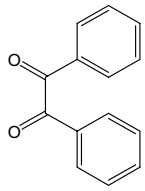
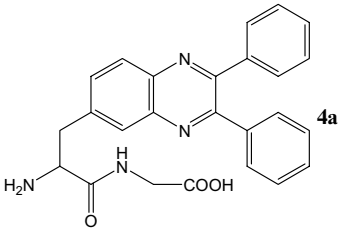
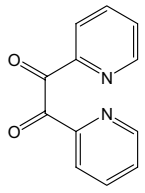
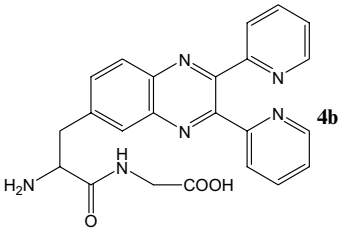
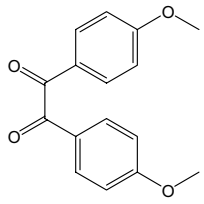
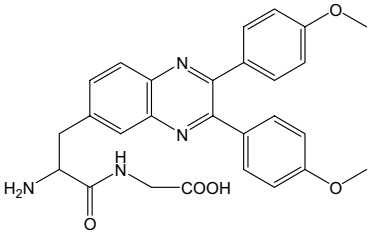
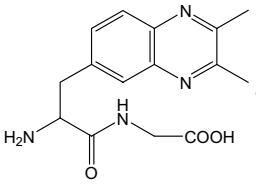
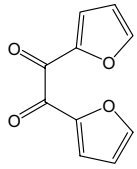
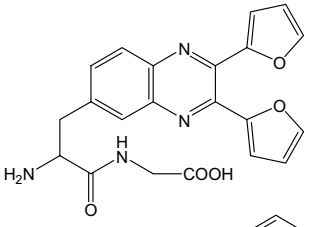
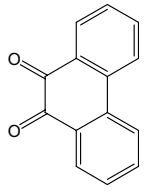
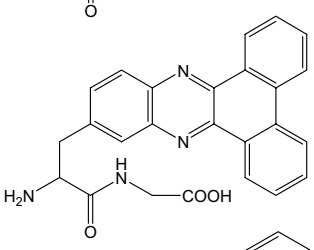
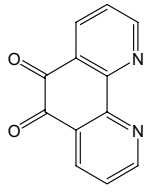
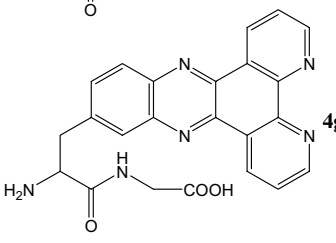
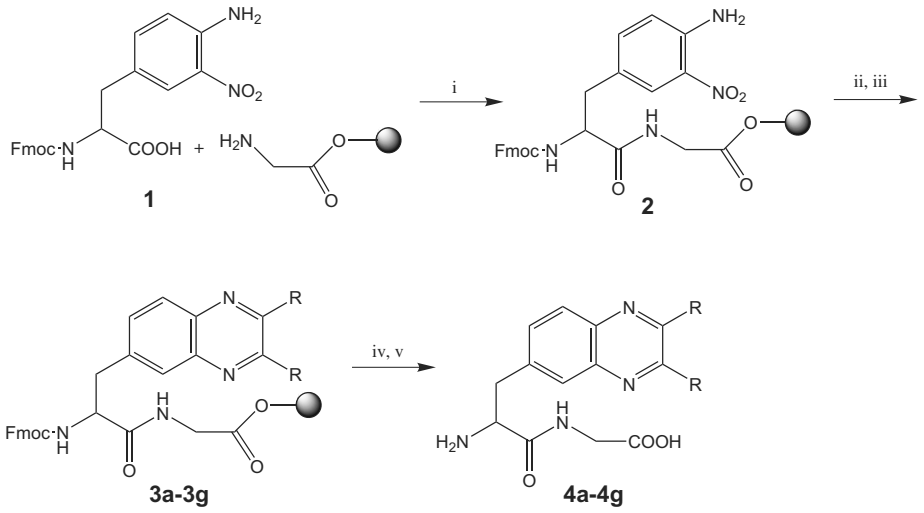
Table 1. Preparation of the quinoxaline-containing peptides

Substrate	Quinoxaline dipeptide	Yield[a] (%)	HPLC[b] purity (%)	M calcd	M+1 found
	 **4a**	93	93	426	427
	 **4b**	90	91	428	429
	 **4c**	91	90	486	487
	 **4d**	93	91	302	303
	 **4e**	82	83	406	407
	 **4f**	97	90	424	425
	 **4g**[c]	94	90	426	427

[a] Yield calculated assuming that the crude product is isolated as a salt containing 2 molecules of TFA.
[b] Purity based on the integral of the crude product absorption at λ 220 nm on RP-HPLC.
[c] Dicarbonyl compound, substrate for **4g** was obtained according to the procedure described in Ref. 15.



Scheme 1. Reagents and conditions: (i) **1** (2 equiv), TBTU (2.0 equiv), DIEA (2.0 equiv) DMF, 2 h at rt; (ii) 2 M $SnCl_2 \times 2H_2O$ in DMF, 48 h at rt; (iii) dicarbonyl compound $(RCO)_2$, (16% solution in DMF) 4 h at rt; (iv) piperidine (20% in DMF) 2×7 min at rt; (v) TFA–water (95:5) 3 h at rt.

loaded with β-(3,4-diaminophenyl)alanine **2**, commercially available Fmoc-Gly-Wang resin was used. After cleavage of the Fmoc protecting group using piperidine, the resulting resin was reacted with Fmoc-Phe(4-NH_2-3-NO_2)-OH **1**, a novel derivative of phenylalanine. Compound **1** was obtained as follows: Ac-Phe(NO_2)-OMe was prepared by esterification of Ac-Phe(NO_2)-OH with methanol catalyzed by an acidic ion exchange resin (Dowex® 50WX4) and then hydrogenation on 10% Pd/C. The resulting Ac-Phe(NH_2)-OMe was acetylated with acetic anhydride. The acetamide (Ac-Phe(NHAc)-OMe) was nitrated with $Cu(NO_3)_2/Ac_2O$ and the product purified on a silica gel column ($CHCl_3$–MeOH 98:2). After acidic hydrolysis (6 N HCl, reflux), the solvent was evaporated and the α-amino Fmoc protecting group was introduced using Fmoc-OSu to yield Fmoc-Phe(4-NH_2-3-NO_2)-OH **1**. The final product was purified by crystallization and its configuration and optical purity were confirmed by crystallography.

The coupling of **1** to the resin was carried out in the presence of TBTU (2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate) and DIEA (*N*,*N*-diisopropylethylamine) in DMF solution. Protection of the aromatic amino group was unnecessary. Treatment of the product with 2.0 M tin(II) chloride dihydrate in DMF at room temperature for 48 h was followed by condensation of the resulting β-(3,4-diaminophenyl)alanine moiety with an α-dicarbonyl compound to give immobilized dipeptides containing a 3,4-disubstituted (quinoxalin-6-yl)alanine residue **3a**–**g**. After removal of the Fmoc protecting group, cleavage of the quinoxaline peptides from the resin was achieved by incubation with trifluoroacetic acid containing 5% of water, according to the standard procedure.[11] A variety of α-dicarbonyl compounds were tested in the above reaction (representative examples are shown in Table 1) employing the optimized conditions. The purity and identity of compounds **4a**–**g** was examined by HPLC, ^{1}H NMR, and ESI-MS.[12] The optical purity of the key intermediate in this synthesis, Fmoc-Phe(4-NH_2-3-NO_2)-OH, was confirmed by crystallography.[13] Synthesis of the peptide bond, deprotection, and the cleavage reaction were performed utilizing standard, racemization-free procedures.[14] Further reactions (reduction of the nitro group and quinoxaline formation) did not involve the stereogenic center (Scheme 1).

The yields of the final crude peptides were in the range 82–97%. Reaction of polymer-bound β-(3,4-diaminophenyl)alanine with glyoxal (data not included in the Table 1) gave a quinoxaline but in poor yield and low purity.

In summary, we have developed an efficient method for the solid-phase synthesis of quinoxaline peptides involving the preparation of a solid-supported peptide containing a β-(4-amino-3-nitrophenyl)alanine residue, reduction with $SnCl_2$, and subsequent reaction of the resultant diamine with a dicarbonyl compound. A standard cleavage procedure gives the desired product. The method is compatible with solid-phase peptide synthesis protocols and could be easily incorporated into combinatorial synthesis. To our knowledge, this method represents the first example of quinoxaline moiety formation in peptides attached to a solid support.

Acknowledgements

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Supplementary data

Details of the synthesis of 4-amino-3-nitrophenylalanine are included. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.06.047.

References and notes

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12. Selected analytical data: Compound **4a**: HPLC: t_R = 34.0 min; ESI-MS *m*/*z* 427 ($[MH]^+$); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.19 (m, 1H), 3.40 (m, 1H), 3.89 (m, 2H), 4.17 (m, 1H), 7.38 (m, 6H), 7.46 (m, 4H), 7.82 (d, 1H, J = 8.6 Hz), 8.08 (s, 1H), 8.12 (d, 1H, J = 8.5 Hz), 8.81 (m, 1H).
    Compound **4b**: HPLC t_R = 21.4 min; ESI-MS *m*/*z* 429 ($[MH]^+$); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.27 (m, 1H), 3.45 (m, 1H), 3.90 (m, 2H), 4.29 (m, 1H), 7.36 (m, 2H), 7.89 (d, 1H, J = 8.6 Hz), 7.97 (m, 4H), 8.14 (s, 1H), 8.18 (d, 1H, J = 8.5 Hz), 8.94 (m, 1H).
    Compound **4c**: HPLC t_R = 34.8 min; ESI-MS *m*/*z* 487 ($[MH]^+$); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.21 (m, 1H), 3.40 (m, 1H), 3.78 (s, 6H) 3.90 (m, 2H), 4.25 (m, 1H), 6.94 (m, 4H), 7.43 (m, 4H), 7.75 (d, 1H, J = 8.6 Hz), 8.02 (s, 1H), 8.05 (d, 1H, J = 8.5 Hz), 8.91 (m, 1H).
    Compound **4d**: HPLC t_R = 19.3 min; ESI-MS *m*/*z* 303 ($[MH]^+$); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 2.66 (s, 6H) 3.15 (m, 1H), 3.35 (m, 1H), 3.88 (m, 2H), 4.20 (m, 1H), 7.65 (d, 1H, J = 8.6 Hz), 7.87 (s,1H), 7.91 (d, 1H, J = 8.5 Hz), 8.88 (m, 1H).
    Compound **4e**: HPLC t_R = 28 min; ESI-MS *m*/*z* 407 ($[MH]^+$).
    Compound **4f**: HPLC t_R = 41.0 min; ESI-MS *m*/*z* 425 ($[MH]^+$); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.30 (m, 1H), 3.48 (m, 1H), 3.92 (m, 2H), 4.32 (m, 1H), 7.82 (m, 2H), 7.89 (m, 2H), 7.92 (d, 1H, J = 8.8 Hz), 8.26 (s, 1H), 8.29 (m, 1H), 8.80 (d, 2H, J = 8.0 Hz), 9.26 (m, 2H) 8.98 (m, 1H).
    Compound **4g**: HPLC t_R = 21.0; ESI-MS *m*/*z* 427 ($[MH]^+$); HPLC: C18 column Vydac: solv. A water + 0.1% TFA, solv. B acetonitrile + 0.1% TFA; gradient 0–100% B in A over 60 min.
13. Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary data no. CCDC 269209. Copies of the data can be obtained free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk.]
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