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Bioorganic Chemistry 33 (2005) 386–392
BIOORGANIC CHEMISTRY
www.elsevier.com/locate/bioorg

Synthesis of sterically hindered peptide analogs using diphenyl phosphite as the coupling reagent

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Received 20 March 2005
Available online 11 July 2005

Abstract

Some model sterically hindered peptide analogs were synthesized with various yields using diphenyl phosphite as the coupling reagent under mild conditions. The experimental procedure is straightforward and the products are easily isolated. This method may be convenient and efficient for the synthesis of hindered peptides.

Keywords: Hindered peptide; Coupling reagent; Diphenyl phosphite; Secondary amine

1. Introduction

Peptides exhibit a wide variety of biological activities [1,2]. Some have also served as drugs or lead compounds in drug development and others have proven useful in studies directed toward the elucidation of biochemical pathways [3,4]. Hence, it is important to develop efficient approaches for the synthesis of peptides.

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doi:10.1016/j.bioorg.2005.05.003

In recent years, peptide coupling reactions have significantly advanced due to the development of efficient coupling reagents [5]. Coupling reagents responsible for the formation of azide, mixed anhydride, and acid halide intermediates have gained substantial popularity in peptide coupling reactions [6]. Other kinds of coupling reagents have also been developed, such as phosphonium reagents [7], uronium reagents [8], immonium reagents [9], carbodiimide reagents [10], imidazolium reagents [11], organophosphorus reagents [12], and acid halogenating reagents [13]. However, peptides isolated from fungi, bacteria, marine sponges, and other lower animal forms, are usually N-methylated and exhibit a variety of biological activities. The chain elongations of these sterically hindered peptides are not readily accomplished using ordinary coupling reagents [14]. The development of efficient coupling reagents for hindered peptides has attracted much attention. Preparation of several simple model peptides was studied using diphenyl phosphite (DPP) and triphenyl phosphite as coupling reagents in the presence of tertiary amines in the 1970s [15,16]. No further progress has not been reported over the past 30 years. Here, we have explored the utility of this reagent (DPP) for the one-pot conversion of acids and sterically hindered amines to amides.

2. Materials and methods

Pyridine was dried over CaH_2 and freshly distilled. ^1H NMR and ^{13}C NMR spectra were recorded on a JOEL JNM-ECA 300 spectrometer using tetramethylsilane as the internal standard. High resolution mass spectra were recorded on a Bruker APEX-II fourier transform ion cyclotron resonance (FT-ICR) MS instrument equipped with 4.7 T super-conduction magnet and an analytical electrospray source.

2.1. General procedure for synthesis of sterically hindered peptides

The acid (1 mmol) and amine (1 mmol) were dissolved in 5 mL of anhydrous pyridine. Subsequently, diphenyl phosphate (2 mmol) in 3 mL of dry pyridine was added dropwise to the solution at 0 °C. The reaction temperature was warmed to room temperature and the stirring was lasted about 1.5 h. The solvent was removed under reduced pressure and purification via silica gel column chromatography provided the corresponding amides using petroleum:ethyl acetate (ratio from 7:1 to 1:3) as eluent.

*2.2. Phenyl-piperidin-1-yl-methanone (**1**)*

^1H NMR (300 MHz, $CDCl_3$): δ 7.37 (s, 5H, aromatic), 3.69 (br, 2H, N–CH_2), 3.32 (br, 2H, N–CH_2), 1.51–1.65 (m, 6H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.00 (CO), 136.12, 129.05, 128.07, 126.46 (–Ph), 48.46, 42.81 ($2 \times$ N–CH_2), 26.18, 25.36, 24.23 ($CH_2CH_2CH_2$). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 190.1232. Found 190.1241.

2.3. Morpholin-4-yl-phenyl-methanone (**2**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (s, 5H, aromatic), 3.47–3.67 (m, 8H, $N(C_2H_4)_2O$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.04 (Ph–CO), 135.00, 129.53, 128.22, 126.76 (–Ph), 66.51 ($2\times$ O–CH_2), 47.86, 42.28 ($2\times$ N–CH_2). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 192.1025. Found 192.1032.

2.4. N-Methyl-N-phenyl-benzamide (**3**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.01–3.31 (m, 10H, aromatic), 3.49 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.59 (CO), 144.71, 135.72, 129.46, 129.00, 128.55, 127.58, 126.74, 126.37 ($2\times$ Ph), 38.27 (CH_3). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 212.1075. Found 212.1092.

2.5. 1-Piperidin-1-yl-ethanone (**4**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.57 (t, 2H, N–CH_2, $^2J = 6.87$ Hz), 3.39 (t, 2H, N–CH_2, $^2J = 6.87$ Hz), 2.12 (s, 3H, CH_3CO), 1.51–1.67 (m, 6H, $CH_2CH_2CH_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.45 (CO), 47.52 (N–CH_2), 42.82 (N–CH_2), 26.26, 25.38, 24.23 ($CH_2CH_2CH_2$), 21.16 (CH_3CO). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 128.1075. Found 128.1089.

2.6. 2-(9H-Fluoren-9-yl)-N-(2-morpholin-4-yl-2-oxo-ethyl)-acetamide (**5**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.74 (m, 8H, Ar of Fmoc), 5.99 (br, s, 1H, NH of Gly), 4.37 (d, 2H, CH_2 of Fmoc, $^2J = 7.23$ Hz), 4.21 (t, 1H, CH of Fmoc, $^2J = 6.87$ Hz), 3.98 (d, 2H, CH_2 of Gly, $^2J = 4.47$ Hz), 3.32–3.61 (m, 8H, $N(C_2H_4)_2O$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.51 (CO of Gly), 156.06 (CO of Fmoc), 143.64, 141.03, 127.49, 126.85, 124.93, 119.76 (Ar mof Fmoc), 66.86 (CH_2 of Fmoc), 66.41, 66.02 ($2\times$ O–CH_2 of morpholinyl), 46.87 (CH of Fmoc), 44.50, 42.29 ($2\times$ N–CH_2 of morpholinyl), 42.01 (Gly-CH_2). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 367.1658. Found 367.1665.

2.7. 2-(9H-Fluoren-9-yl)-N-[(methyl-phenyl-carbamoyl)-methyl]-acetamide (**6**)

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.15–7.73 (m, 13H, Ar of Fmoc and Ph), 5.85 (br s, 1H, Gly-NH), 4.31 (d, 2H, CH_2 of Fmoc,$^2J = 7.23$ Hz), 4.18 (t, 1H, of CH of Fmoc, $^2J = 6.87$ Hz), 3.71 (s, 2H, CH_2 of Gly), 3.27 (s, 3H, N–CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 168.13 (CO of Gly), 156.01 (CO of Fmoc), 143.74, 141.69, 141.06, 130.01, 128.45, 127.48, 126.99, 126.87, 124.99, 119.76 (Ar of Fmoc and Ph), 66.86 (CH_2 of Fmoc), 46.95 (CH of Fmoc), 43.19 (CH_2 of Gly), 37.33 (N–CH_3). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 387.1609. Found 387.1622.

*2.8. 2-(9H-Fluoren-9-yl)-N-[2-methyl-1-(morpholine-4-carbonyl)-propyl]-acetamide (**7**)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17–7.76 (m, 8H, Ar of Fmoc), 5.78 (br, d, 1H, NH of Val, $^2J = 8.94$ Hz), 4.18–4.50 (m, 4H, CH of Val, CH and CH_2 of Fmoc), 3.48–3.72 (m, 8H, $N(C_2H_4)_2O$), 1.92–2.03 (m, CH of Val), 0.97 (d, 3H, CH_3 of Val, $^2J = 6.87$ Hz), 0.93 (d, 3H, CH_3 of Val, $^2J = 6.87$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.46 (Val–CO), 156.40 (CO of Fmoc), 143.67, 141.16, 127.59, 126.95, 124.99, 119.86 (Ar of Fmoc), 66.99 (CH_2 of Fmoc), 66.67, 66.51 ($2 \times$ O–CH_2 of morpholinyl), 55.10 (CH of Val), 47.05 (CH of Fmoc), 46.23, 42.34 ($2 \times$ N–CH2 of morpholinyl), 31.43 (CH of Val), 19.47, 17.27 ($2 \times CH_3$ of Val). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 409.2127. Found 409.2141.

*2.9. 2-(2-9H-Fluoren-9-yl-acetylamino)-3,N-dimethyl-N-phenyl-butyramide (**8**)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.21–7.73 (m, 13H, Ar of Fmoc and Ph), 5.67 (br, d, 1H, NH of Val, $^2J = 9.27$ Hz), 4.17–4.41 (m, 4H, CH of Val, CH and CH_2 of Fmoc), 3.27 (s, 3H, N–CH_3), 1.81–1.91 (m, 1H, CH of Val), 0.83 (d, 3H, CH_3 of Val, $^2J = 6.87$ Hz), 0.74 (d, 3H, CH_3 of Val, $^2J = 6.87$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.85 (CO of Val), 155.87 (CO of Fmoc), 143.69, 142.51, 141.23, 129.58, 128.00, 127.43, 127.28, 126.80, 124.98, 119.72 (Ar of Fmoc and Ph), 66.63 (CH_2 of Fmoc), 55.93 (CH of Val), 46.98 (CH of Fmoc), 37.51 (N–CH_3), 31.39 (CH of Val), 19.32, 17.15 ($2 \times CH_3$ of Val). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 429.2178. Found 429.2188.

*2.10. 2-(9H-Fluoren-9-yl)-N-[1-(morpholine-4-carbonyl)-2-phenyl-propyl]-acetamide (**9**)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.84 (m, 13H, Ar of Fmoc and Phe), 6.04–6.10 (m, 1H, NH of Phe), 4.92–5.02 (m, 1H, CH of Phe), 4.38–4.51 (m, 2H, CH_2 of Fmoc), 4.26 (t, 1H, CH of Fmoc, $^2J = 6.87$ Hz), 2.89–3.66 (m, 10H, CH_2 of $N(C_2H_4)_2O$ and Phe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.92 (CO of Phe), 155.68 (CO of Fmoc), 143.66, 141.15, 135.83, 129.44, 128.54, 127.60, 127.14, 126.94, 124.98, 119.87 (Ar of Fmoc and Phe), 67.00 (Fmoc-CH_2), 66.23, 65.83 ($2 \times$ O–CH_2 of morpholinyl), 51.16 (CH of Phe), 46.97 (CH of Fmoc), 45.87, 42.17 ($2 \times$ N–CH_2 of morpholinyl), 40.12 (CH_2 of Phe). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 457.2127. Found 457.2136.

*2.11. 2-(2-9H-Fluoren-9-yl-acetylamino)-N-methyl-3,N-diphenyl-butyramide (**10**)*

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.82–7.75 (m, 18H, Ar of Fmoc, Phe and Ph), 5.65 (br, s, 1H, NH of Phe), 4.60 (m, 1H, CH of Phe), 4.15–4.36 (m, 3H, CH and CH_2 of Fmoc), 3.22 (s, 3H, N–CH_3), 2.72–2.97 (m, 2H, CH_2 of Phe). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.53 (CO of Phe), 155.39 (CO of Fmoc), 143.82, 142.33, 141.21, 136.28, 129.69, 129.33, 128.38, 127.60, 127.23, 126.98, 126.80, 125.17, 125.10, 119.90 (Ar of Fmoc, Phe and Ph), 66.86 (CH_2 of Fmoc), 52.69 (CH of Phe), 47.06 (CH of Fmoc),

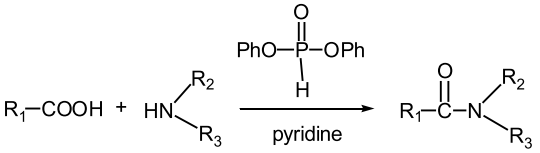
39.64 (CH_2 of Phe), 37.54 (N–CH_3). Positive ion HR-ICRESI-MS: $[M+H]^+$ *m/z* Cacld 477.2178. Found 477.2192.

3. Results and discussion

The synthetic route for sterically hindered peptide analogs is summarized in Scheme 1. The reaction of *N*-[(9H-fluoren-9-yl-methoxy)carbonyl]-glycine (Fmoc-Gly) and *N*-methyl phenylamine (entry 6 in Table 1) was carried out in the presence of DPP. Accordingly, an equivalent of Fmoc-Gly and *N*-methyl phenylamine were dissolved in dry pyridine, and then two equivalents of DPP in anhydrous pyridine were added dropwise to the solution at 0 °C. The solution was warmed to room temperature. The purpose for the addition of excess DPP was to remove the trace amount of water in the solution. About 1.5 h later, TLC showed that the starting materials were quantitatively transferred into Fmoc-Gly-N(Me)-Ph, and the target product Fmoc-Gly-N(Me)-Ph was obtained in excellent yield (96%) after isolation using silica gel column chromatography. Encouraged by the promising result, we successfully synthesized a series of amides through reaction of various acids and secondary amines in the presence of DPP. Their coupling also gave yields ranging from 65 to 95% as shown in Table 1. The structures of the target products were determined by ^1H, ^{13}C NMR spectroscopy and ESI-MS.

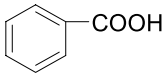
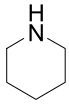
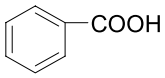
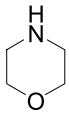
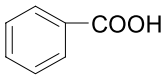
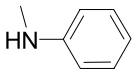
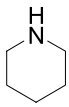
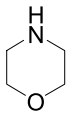
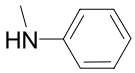
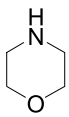
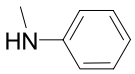
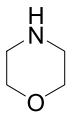
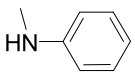
Compared with other kinds of coupling reagents, DPP has five advantages. The method provides reasonably high yields for the synthesis of the hindered peptides and the experimental procedure results in a high regioselectivity. Reaction of the acid with DPP in pyridine could lead to a phosphonic-carboxylic mixed anhydride intermediates and the nucleophilic attack of the amino group could occur on the carbonyl rather than on the phosphorus in the anhydrides to give an amidate. Also, the excess coupling reagent (diphenyl phosphite, ^{31}P NMR 2.0 ppm) and its byproduct (phenyl phosphite, ^{31}P NMR 1.6 ppm) are easily removed during purification. Fourth, the coupling reaction can be carried out by a one-pot procedure. For some organophosphorus reagents such as diphenylphosphorochloridate (DPP-Cl), the carboxylic acid was reacted first and the amine cannot be added to the resulting solution until DPP-Cl is used up. Finally, the coupling reagent DPP is inexpensive, and the coupling of hindered peptides occurs fairly rapid.

In conclusion, some sterically hindered peptide analogs were synthesized using diphenyl phosphite as the coupling reagent. The method is straightforward, occurs



Scheme 1. Synthetic route for amides using diphenyl phosphite as the coupling agent.

Table 1
Reaction yields of acids and amines in the coupling of diphenyl phosphite

Entry	R_1-COOH	R_2-NH-R_3	Yield (%)
1			65
2			95
3			65
4	CH_3COOH		92
5	Fmoc-Gly		93
6	Fmoc-Gly		96
7	Fmoc-Val		93
8	Fmoc-Val		92
9	Fmoc-Phe		88
10	Fmoc-Phe		90

under mild conditions, and the desired products are readily purified. Hence, it may find use for synthesis of hindered peptides on a large scale.

Acknowledgments

This work was supported by the Excellent Dissertation Foundation of the Chinese Ministry of Education (No. 200222), the Excellent Young Teacher Program of MOE,

P.R.C., and the National Natural Science Foundation of China (Grant No. 20472042).

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