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Synthesis of dipeptides from *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione activated α-amino acids

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ABSTRACT

A simple two step procedure for the synthesis of a dipeptide from *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione (HO-ASUD) activated α-amino acids is described. In presence of DCC, *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione readily esterifies the carboxylic acid group of all the N-protected amino acids to yield crystalline *N*-hydroxy-3-aza spiro[5,5]undecane-2,4-dione activated carboxy ester. The *N*-hydroxy-3-aza spiro[5,5]undecane-2,4-dione activated carboxy esters of N-protected amino acids readily condensed with other amino acids and gave a dipeptide. This new method is effective for the DCC coupling of a variety of chiral amino acids without loss of enantiomeric purity. Synthesis of fifteen dipeptides including the hitherto unreported Fmoc-L-Orn(Boc)-Val-OMe, Fmoc-L-Cys(trt)-Gly-OEt and Boc-L-Tyr-Gly-OEt is presented.

1. Introduction

Peptide synthesis is one of the most important and widely studied chemical transformations in organic synthesis. Suitability of the protecting and activating groups[1–5] is one of the major deterrent problems that necessitate modification of the prevailing peptide methodologies.

There are numerous reports on the use of activated spiro esters in peptide synthesis.[6–11] The *N*-hydroxy compounds used to activate the N-protected amino acids were NOSU (*N*-hydroxysuccinimide)[12–14] 1-hydroxybenzotriazole[15] (HOBT) and *N*-hydroxyphthalimide (HOPh).[16] Recently, it was observed that HOBT is not safe to use under ambient conditions. In the present investigation, we report a facile two step synthesis of fifteen dipeptides including the hitherto unreported Fmoc-L-Orn(Boc)-Val-OMe, Fmoc-L-Cys(trt)-Gly-OEt and Boc-L-Tyr-Gly-OEt involving *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione (OH-ASUD) activated amino acid esters as intermediates and DCC as a coupling reagent in THF.

2. Results and discussion

The HO-ASUD is a stable reagent. It was prepared starting from cyclohexanone following Stevens et al.[17] A typical procedure for the synthesis of activated Boc-alanine–ASUD ester is shown in Scheme 1. When equimolar amounts of Boc-alanine and HO-ASUD are stirred overnight in THF at room temperature in the presence of 1.1 equiv of DCC, its activated ester, Boc-alanine–ASUD, is obtained. Similarly conversion of Fmoc, Boc and Z-protected amino acids to their respective ASUD esters was carried out by stirring equimolar amounts of the protected amino acids and HO-ASUD overnight at room temperature in the presence of DCC in THF. Similarly the hydroxyl group of HO-ASUD can also readily be esterified with N- and side-chain-protected amino acids. Therefore, formation of a protected amino acid-ASUD ester using DCC occurs with all of the amino acids. During the course of the reaction, the reaction mixture developed a white suspension due to the formation of insoluble dicyclohexylurea. The activated amino acid spiro ester is stable and readily crystallizes, during which process the unreacted HO-ASUD was removed. The formation of activated amino acid-ASUD ester can be readily monitored by TLC as well as by the appearance of a new band around 1745 cm^{-1} in the infrared spectrum, due to the spiro ASUD ester.

It was found that esterification of N-protected L-amino acids with HO-ASUD can be carried out not only in THF but also in other organic solvents such as acetone, dichloromethane, chloroform, toluene and acetonitrile, but not in *n*-hexane and water.

When the activated amino acid-ASUD ester is charged and stirred overnight with equimolar amounts of another α-amino acid in water–THF (1:1 v/v) and 2.5–3.0 equiv of Na_2CO_3 at room temperature, an N-protected dipeptide was obtained. Similarly an N-protected dipeptide methyl ester was prepared using 2.5 equiv of triethylamine in place of Na_2CO_3 in THF at room temperature along the lines of Scheme 1. The overall yield of dipeptides obtained is very high ranging between 69% and 86% as shown in Table 1. No premature deblocking of the N-protecting and other side chain groups occurred either during esterification or peptide bond

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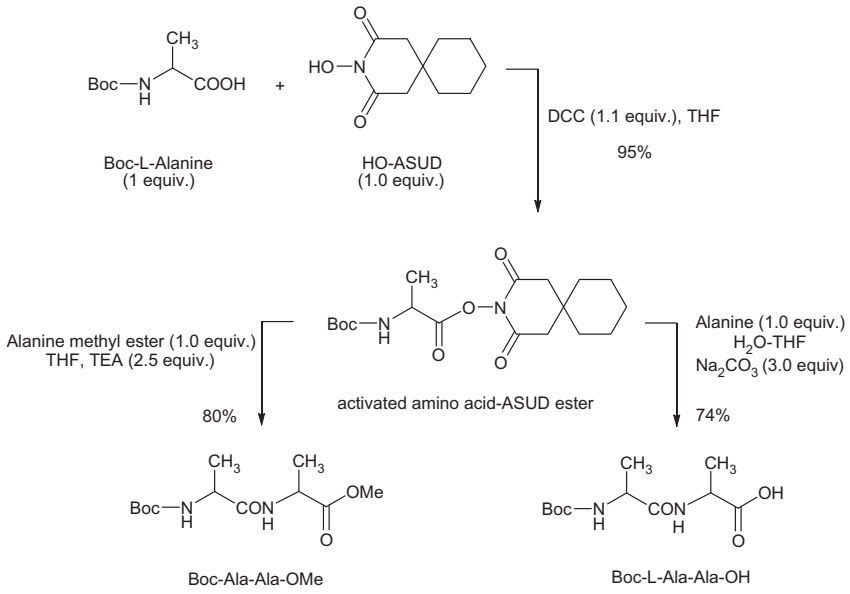

Scheme 1. Synthesis of Boc-Ala-Ala-OH and Boc-Ala-Ala-OMe involving *N*-hydroxy-3-azaspiro[5,5]undecane-2,4-dione activated amino acid ester intermediate.

Table 1
Dipeptides prepared involving the activated amino acid-ASUD ester intermediate

S. No.	Product	Obtained yield	Reported yield	Obtained ORD[b]	Reported ORD *c* 1 $[\alpha]_D^{20}$
1	Z-Gly-Gly-OEt	76	60[20]	—	—
2	Fmoc-Gly-Val-OMe	88	89[21]	+18.2	+18.6 ($CHCl_3$)
3	Z-L-Pro-Val-OH	80.5	78[22]	−12.6	−13.4 (DMF)
4	Boc-L-Ala-Ala-OMe	80	66.7[23a]	−50.8	−51.5 (MeOH)
5	Boc-L-Ala-Ala-OH	74	57[23a]	−42.6	−43.1 (MeOH)
6	Boc-L-Phe-Gly-OEt	71	78[20]	−4.7	−4.2 (EtOH)
7	Z-L-Trp-Ala-OH	83	77[23b]	−38.5	−40.1 (DMF)
8	Z-L-Gly-Leu-OH	69	56[20]	−18.3	−18.2 (1 N NaOH)
9	Fmoc-L-Orn(Boc)-Val-OMe[a]	78	New	−29.2 (MeOH)[c]	—
10	Boc-L-Lys(Z)-Gly-OMe	86	83[13]	−12.9	−13.1 (MeOH)
11	Boc-L-Pro-Pro-OH	72	74[23c]	−123.2	−121.5 (MeOH)
12	Fmoc-L-Cys(trt)-Gly-OEt[a]	84	New	+34.7 (MeOH)[d]	—
13	Z-L-Glu(OMe)-Dihydroxy-Tyr-OMe	78.6	69[23d]	−8.3	−8.5 (MeOH)
14	Boc-Gly-Pro-OH	76.4	71[23e]	−22.4	−23.9 (EtOH)
15	Boc-L-Tyr-Gly-OEt[a]	75	New	+3.3(MeOH)[e]	—

[a] New peptides fully characterized by ^1H NMR, ^{13}C NMR, IR and Mass spectra.
[b] Observed values are obtained under the identical conditions of the reported literature conditions. ORD = $[\alpha]_D^{20}$ (*c* 10 mg/ml, CH_3OH).
[c] Chiral HPLC: Chiral PACK-AS-H; *n*-Hexane/IPA/TFA (50:50:0.1%); %ee: 98.29.
[d] Chiral HPLC: Chiral PACK-AS-H; *n*-Hexane/IPA/TFA (80:20:0.1%); %ee: 99.78.
[e] Chiral HPLC: Chiral PACK-AD-H; *n*-Heptane/IPA/TFA (90:10:0.1%); %ee: 99.96.

formation. The co-product HO-ASUD was washed off with 5% Na_2CO_3 solution. A control experiment was performed using the N-protected amino acid-*N*-hydroxy succinimide method[18] for TLC analysis and enantiomeric purity. The purity of the N-protected dipeptides was monitored by HPLC[19] and compared with that of the respective peptides obtained by the N-OSU method. The structure of all the dipeptides prepared was characterized by micro analysis, IR, NMR and Mass spectra and compared with the reported literature. The IR spectrum correlated more than 95% with standard samples. All of the dipeptides prepared by the present method and NOSU method are listed in Table 1. Entries 9, 12 and 15 in the table are new dipeptides, which are hitherto unreported. Their structure is established by mass, ^1H NMR, ^{13}C NMR and infra red spectra. The ^{13}C NMR has peaks at 169, 165 and 156 ppm, respectively for the newly formed peptide carbonyl carbon, respectively. The proton NMR spectra of Fmoc-L-Orn(Boc)-Val-OMe, Fmoc-L-Cys(trt)-Gly-OEt and Boc-L-Tyr-Gly-OEt exhibited new signals at 8.3, 9.07 and 9.04 ppm, respectively representing the newly formed peptide proton. Similarly the pseudo molecular ions obtained at 689 (M^++H_2O) and 365 (M−1) in the ES Mass confirms the structure of 12 and 15. In the case of entry 9, no molecular ion peak in the mass spectrum was obtained. However the fragment obtained at *m/z* 512 is the ion obtained after the loss of an OMe and the side chain of valine.

The present method of dipeptide synthesis has the following merits. Preparation of HO-ASUD is simple. It has shelf stability at room temperature and is not hygroscopic like N-OSU. It is highly soluble in most of the organic solvents employed in peptide synthesis. It retains all the advantage of DCC coupled reactions. HO-ASUD is more reactive than N-OSU and gave comparable yields in less time. The overall yield of dipeptide with the present method and with the N-OSU as activating group under identical conditions is shown in Table 1. It can be noticed from Table 1 that HOASUD activation is comparable with the N-OSU method. The

enantiomeric purity was measured by optical rotatory dispersion (ORD) and compared with standard samples.[18] It has been found that the dipeptide formation occurred with complete preservation of stereochemistry. HO-ASUD activation of protected amino acids is not sensitive to any particular protecting group or amino acid and can be widely employed in peptide synthesis.

3. Conclusion

Synthesis of N-protected dipeptides and N-protected dipeptide methyl esters via N-protected L-amino acid activated ASUD ester intermediates is simple and can be carried out not only in THF but also in other organic solvents such as acetone, dichloromethane, toluene and acetonitrile. The overall yield of the dipeptides obtained is high and comparable to that of N-OSU.[12] We have prepared three new dipeptides involving these activated ASUD ester intermediates.

4. Experimental

4.1. Preparation of Boc-L-alanine–ASUD ester

Boc-L-alanine 1.0 g (0.0052 mol), OH-ASUD 1.04 g (0.00529 mol), DCC 1.19 g (0.0058 mol) and THF (25 ml) were stirred in a 100 ml RB flask at room temperature for 12 h. The dicyclohexylurea was filtered and the filtrate was concentrated to give the Boc-L-alanine-ASUD ester. Yield 1.9 g (100%).

4.2. Preparation of Boc-L-Ala-ala-OH 5

At first, L-alanine 0.45 g (0.00516 mol) and Na_2CO_3 1.64 g (0.01548 mol) were dissolved in 16 ml of water at room temperature in a 100 ml three necked round bottomed flask. Boc-L-alanine-ASUD ester 1.9 g (0.00516 mol) dissolved in 16 ml of THF was added to the above reaction mixture at room temperature over a period of 5 min. After the addition was complete, the reaction mixture was stirred for 10 h at room temperature. The reaction mixture was acidified with 5% $KHSO_4$ to reach a pH of 2–3.The product was extracted into ethyl acetate. The ethyl acetate extract was washed with 5% Na_2CO_3 solution, water, brine solution and dried over anhydrous Na_2SO_4. The ethyl acetate was removed at reduced pressure. The oily crude product obtained was crystallized using ethyl acetate and *n*-hexane. The yield of white crystalline Boc-L-Ala-Ala-OH is 0.98 g (74%).

4.3. Preparation of Boc-L-Ala-Ala-OMe 4

At first, Boc-L-alanine-ASUD ester 1.9 g (0.00516 mol), L-alanine methyl ester hydrochloride 0.71 g (0.00516 mol), triethylamine 1.30 g (0.0129 mol) and THF 20 ml were charged into a 100 ml three necked round bottomed flask at room temperature. The reaction mixture was stirred over night at room temperature. The triethylamine hydrochloride salt was filtered and the filtrate was concentrated under vacuum to give an oily crude. The oily crude was dissolved in ethyl acetate and washed with 0.5% $NaHCO_3$ solution and water. The ethyl acetate layer was dried over Na_2SO_4 and distilled under vacuum to give an oily crude product. The yield of Boc-L-Ala-Ala-OMe is 1.1 g (80%).

4.4. Preparation of Fmoc-L-Orn(Boc)-ASUD ester

At first, Fmoc-L-Orn(Boc)-OH 1.0 g (0.0022 mol), OH-ASUD 0.43 g (0.0022 mol), DCC 0.45 g (0.0022 mol) and THF 10 ml were stirred in a 50 ml three necked round bottomed flask at room temperature for 12 h. The dicyclohexylurea was filtered and concentrated to give a colourless viscous oily liquid of Fmoc-L-Orn(Boc)-ASUD ester. Yield 1.3 g (100%).

4.5. Preparation of Fmoc-L-Orn(Boc)-Val-OMe 9

L-Valine methyl ester 0.27 g (0.002 mol) and Na_2CO_3 0.63 g (0.006 mol) were dissolved in 16 ml of water at room temperature in a 100 ml three necked round bottomed flask. Next, Fmoc-L-Orn(-Boc)-ASUD ester 1.3 g (0.002 mol) dissolved in 16 ml of THF was added to the above reaction mixture at room temperature over a period of 5 min. After the addition was complete, the reaction mixture was stirred for 10 h at room temperature. The reaction mixture was acidified with 5% $KHSO_4$ to reach a pH of 2–3. The product was extracted into ethyl acetate. The ethyl acetate extract was washed with 5% Na_2CO_3 solution, water, brine solution and dried over anhydrous Na_2SO_4. The ethyl acetate was removed at reduced pressure. The oily crude product obtained was crystallized using ethyl acetate and *n*-hexane. The yield of pale yellow crystalline Fmoc-L-Orn(Boc)-Val-OMe is 0.9 g (78%). Mp: 155–157 °C, ^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ 7.1–7.7 (m, 8H), δ 5.92 (d, 1H), δ 5.5 (dd, 1H), δ 4.1–4.8 (m, 2H), δ 3.37 (s, 3H), δ 3.0 (d, 1H), δ 2.5–2.8 (m, 2H), δ 1.9 (m, 2H), δ 1.7 (m, 2H), δ 1.50–1.59 (s, 9H), δ 0.77–0.98 (dd, 6H); ^{13}C NMR (75 MHz,$CDCl_3$-*d*): δ 168.93, 168.69, 157.45, 156.43, 144.04, 141.22, 127.69, 127.08, 125.17, 119.9, 79.1, 77.5, 76.66, 67.2, 54.6, 49.2, 47.1, 44.0, 39.8, 36.6, 35.8, 33.7, 29.8, 28, 26.9, 18.1; IR (cm^{-1}): 3332.56, 2930.63, 2853.36, 164.71, 1627.82; MS *m/z* 512.2 (M−OMe and side chain of valine). $[\alpha]_D^{20} = -29.2$ (*c* 1, CH_3OH); Chiral HPLC: Chiral PACK-AS-H; *n*-Hexane/IPA/TFA (50:50:0.1%); %ee: 98.29; Elemental analysis; observed (theoretical): C = 65.13 (65.59); H = 7.21 (7.28); N = 7.35 (7.4).

4.6. Preparation of Fmoc-L-Cys(trt)- ASUD ester

Fmoc-L-Cys(trt)-OH 1.5 g (0.0025 mol), OH-ASUD 0.5 g (0.0025 mol), DCC 0.52 g (0.0025 mol) and THF (15 ml) were stirred in a 50 ml three neck round bottomed flask at room temperature for 12 h. The dicyclohexylurea was filtered and the filtrate concentrated to give Fmoc-L-Cys(trt)-ASUD ester. Yield 1.71 g (100%).

4.7. Preparation of Fmoc-L-Cys(trt)-Gly-OEt 12

Glycine ethyl ester 0.21 g (0.0022 mol) and Na_2CO_3 0.7 g (0.0066 mol) were dissolved in 20 ml of water at room temperature in a 100 ml three neck round bottomed flask. Fmoc-L-Cys(trt)-ASUD ester 1.71 g (0.0022 mol) dissolved in 20 ml of THF was added to the above reaction mixture at room temperature over a period of 5 min. After the addition was complete, the reaction mixture was stirred for 10 h at room temperature. The reaction mixture was acidified with 5% $KHSO_4$ to reach a pH to 2–3. The product was extracted into ethyl acetate. The ethyl acetate extract was washed with 5% Na_2CO_3 solution, water, brine solution and dried over anhydrous Na_2SO_4. The ethyl acetate was removed at reduced pressure. The oily crude product obtained was crystallized using ethyl acetate and *n*-hexane. The yield of white crystalline Fmoc-L-Cys(trt)-Gly-OEt is 1.25 g (84%). Mp: 78–82 °C, ^{1}H NMR (300 MHz, $CDCl_3$-*d*): 7.1–7.4 (m, 24H), δ 4.6 (s, 1H), δ 4.2 (s, 2H), δ 4.1 (d, 1H), δ 3.5 (m, 4H), δ 2.8–3.1 (dd, 2H), δ 1.7 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$-*d*): δ 178.0, 165.47, 156.99, 144.49, 144.10, 129.49, 128.16, 127.98, 127.89, 127.05, 126.87, 77.5, 77.10, 76.67, 67.53, 53.19, 49.05,36.1, 29.03, 26.4, 14.1; IR (cm^{-1}): 3327.94, 3056.65, 2928.03, 2851.32, 1681.75, 1627.14; MS *m/z* 689 (M+H_2O). $[\alpha]_D^{20} = +34.7$ (*c* 1, CH_3OH); Chiral HPLC: Chiral PACK-AS-H; *n*-Hexane/IPA/TFA (80:20:0.1%); %ee: 99.78; Elemental

analysis; observed (theoretical): C = 72.97 (73.41); H = 5.64 (5.71); N = 4.15 (4.18).

4.8. Preparation of Boc-L-Tyr- ASUD ester

Boc-L-tyrosine 2.0 g (0.0071 mol), OH-ASUD 1.4 g (0.0071 mol), DCC 1.46 g (0.0071 mol) and THF 25 ml were stirred in a 100 ml three neck round bottomed flask at room temperature for 12 h. The dicyclohexylurea was filtered and the filtrate concentrated to give Boc-L-tyrosine-ASUD ester. Yield 3.2 g (100%).

4.9. Preparation of Boc-L-Tyr-Gly-OEt 15

Glycine ethyl ester 0.71 g (0.0069 mol) and Na_2CO_3 2.2 g (0.01548 mol) were dissolved in 25 ml of water at room temperature in a 100 ml three neck round bottomed flask. Boc-L-tyrosine-ASUD ester 3.2 g (0.00516 mol) dissolved in 25 ml of THF was added to the above reaction mixture at room temperature over a period of 5 min. After the addition was complete, the reaction mixture was stirred for 10 h at room temperature. The reaction mixture was acidified with 5% $KHSO_4$ to reach a pH of 2–3.The product was extracted into ethyl acetate. The ethyl acetate extract was washed with 5% Na_2CO_3 solution, water, brine solution and dried over anhydrous Na_2SO_4. The ethyl acetate was removed at reduced pressure. The oily crude product obtained was crystallized using ethyl acetate and *n*-hexane. The yield of off-white crystalline Boc-L-Tyr-Gly-OEt is 1.9 g (75%). Mp: 122–125 °C, ^{1}H NMR (300 MHz, $CDCl_3$-*d*): δ 7.04–7.06 (dd, 2H), δ 6.74–6.77 (dd, 2H), δ 6.51–6.54 (t, 1H), δ 5.1 (m, 1H), δ 4.4 (d, 1H), δ 4.17–4.24 (q, 2H), δ 3.95–4.02 (d, 2H), 3.00–3.02 (d, 2H), δ 1.38–1.46 (s, 9H), δ 1.26–1.28 (t, 3H); ^{13}C NMR (75 MHz,$CDCl_3$-*d*): δ 172.28, 169.62, 157.40, 155.69, 155.53, 130.32, 127.48, 115.64, 80.44, 77.5, 77.0, 76.65, 61.60, 55.85, 49.18, 41.35,37.61, 33.82, 28.24, 25.53, 24.56, 14.061; IR (cm^{-1}): 3335.50, 2982.98, 2928.95, 1743.08, 1690.05, 1638.92, 1595.19; MS *m/z* 365.13 (M−1). $[\alpha]_D^{20} = +3.3$ (*c* 1, CH_3OH); Chiral HPLC: Chiral PACK-AD-H; *n*-Heptane/IPA/TFA (90:10:0.1%); %ee: 99.96; Elemental analysis; observed (theoretical): C = 58.82 (59.0); H = 7.11 (7.15); N = 7.60 (7.65).

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