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A novel kinetically-controlled peptide synthesis — dramatic increase of chemical yield with retention of chiral integrity

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Abstract

Peptide synthesis employing the highly selective reaction of isobutyl chloroformate at the carboxyl group of the *N*-protected amino acid, almost to the exclusion of the amino group of the *C*-protected amino acid, is described. This one-stage, kinetically-controlled strategy remarkably affords peptides with excellent optical purity in high chemical yields. © 1999 Elsevier Science Ltd. All rights reserved.

Conventional peptide synthesis requires the activation of the carboxyl group of *N*-protected amino acid prior to coupling with the amino group of a *C*-protected amino acid.[1] One widely used activation reagent, isobutyl chloroformate (IBCF), was initially reported by Vaughan[2] more than fifty years ago. His two-stage methodology begins with the formation of a mixed anhydride by treating an *N*-protected amino acid with IBCF in the presence of a tertiary amine base. The *C*-protected amino acid is then added and peptide formation is generally completed within an hour. Although this methodology is popular for its convenience and economy, problems associated with racemization and low conversions have often been reported.[3] We describe herein a simple concept that affords peptides with excellent optical purity and high chemical yield, especially for hindered peptides.

In the present study, we explored a novel kinetically-controlled synthetic strategy (Scheme 1). This is a one-stage procedure involving the activation of an *N*-protected amino acid $\mathbf{AA_1}$ with IBCF in the presence of a *C*-protected amino acid $\mathbf{AA_2}$ and a tertiary amine base. This protocol, if successful, could potentially shorten the lifetime of the activated intermediate **C** in solution and eliminate one of the major factors that cause the racemization of an asymmetric center (**E->F**).[4] In addition, this could minimize the accumulation of the symmetric anhydride **G**, which is responsible for the recovery of the unreacted amino acid $\mathbf{AA_1}$ in the conventional two-stage method.[5] In our concept, any symmetric anhydride formed during the activation process can react with *C*-protected amino acid $\mathbf{AA_2}$ to afford the desired peptide **D** and one equivalent of *N*-protected amino acid $\mathbf{AA_1}$. The unreacted $\mathbf{AA_1}$ can then immediately be recycled to generate more mixed anhydride **C** and subsequently more product **D**. Consequently, full consumption

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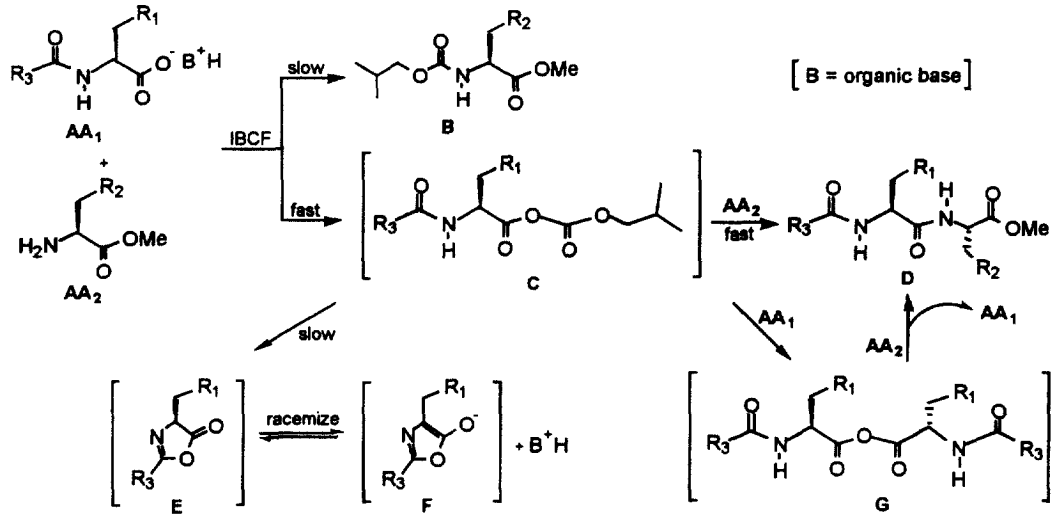
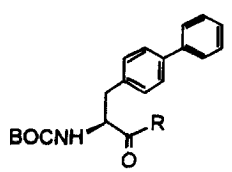
of the *N*-protected amino acid AA_1 can be achieved. This methodology will only be successful if, under the conditions employed, the carboxylate ion of AA_1 reacts much faster than the primary amino group of AA_2 does with IBCF. Otherwise, the undesired carbamate **B** will be the dominant product. The results compiled in Table 1 clearly illustrate the effectiveness of this strategy (Procedure A) over the conventional two-stage protocol (Procedure B).

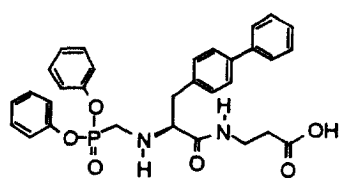


Scheme 1. Proposed mechanism for the kinetically-controlled peptide synthesis

The proof of superiority in chemical yield using this one-stage procedure was demonstrated in the synthesis of Boc-4-Ph-Phe-β-Ala-OBzl (**1**, entry 1), an important intermediate for a potential antihypertensive drug CGS 25462 (**3**).[6] Formation of peptide **1** from amino acid **2**[7] was monitored by HPLC and found to be instantaneous upon the addition of isobutyl chloroformate. After completion of the addition, >99% consumption of **2** was achieved. The colorless solid **1** was obtained in 95% yield from **2** and determined to be enantiomerically pure by HPLC (optical purity: 99.4%; chemical purity: 97.5%).[8] The only by-product generated from this procedure was an oily carbamate (*i*-BuOCONHCH$_2$CH$_2$COOCH$_2$C$_6$H$_5$, **4**, 5%), which was easily separated from crystalline **1** during the process of filtration. By comparison, the conventional two-stage procedure resulted in unreacted **2** (20%) and afforded **1** (75%) contaminated with 2.5% of unwanted enantiomer (entry 2). Coupling of racemization-tolerant amino acids at higher temperatures also gave product in excellent enantiomeric purity (entry 3). Increasing the addition time of IBCF from 1.5 to 5 h resulted in no significant decrease in racemization (entry 4). Excellent chemical yields were consistently obtained employing the one-stage procedure for peptide synthesis involving a hindered amino acid (Val-OMe) regardless of which enantiomeric amino acids it reacted with (entries 5–8).



1, R = NHCH$_2$CH$_2$COOCH$_2$C$_6$H$_5$
2, R = OH



3, CGS 25462

Improvements in optical purity for hindered peptides are evident also from the results shown in Table 1. The most striking example of the effectiveness of this one-stage methodology involves the coupling of racemization sensitive benzoylvaline with valine methyl ester.[9] A remarkable decrease in the amount

Table 1
Summary of kinetically-controlled peptide synthesis

Entry	R_1COOH	R_2NH_2 salt	Procedure[a,b]	Racemization (%)[c,d], isomer	yield (%)
1	2	β-Ala-OBzl[e]	A	0.6, D-	95
2	2	β-Ala-OBzl[e]	B	2.5, D-	75
3	2	β-Ala-OBzl[e]	A[f], 25 °C	0.6, D-	95
4	2	β-Ala-OBzl[e]	A[g], 5 hr addn	0.5, D-	95
5	Z-Phe	Val-OMe HCl	A	<0.1, DL-	93[h]
6	Z-D-Phe	Val-OMe HCl	A	<0.1, LL-	91
7	Z-Ala	Val-OMe HCl	A	<0.1, DL-	88[i]
8	Z-D-Ala	Val-OMe HCl	A	<0.1, LL-	91
9	Z-Phe-Val	Ala-OMe HCl	A	0.1, LDL-	90
10	Z-Phe-Val	Ala-OMe HCl	B	10, LDL-	80
11	Z-Phe-D-Val	Ala-OMe HCl	A	0.1, LLL-	91
12	Bz-Val	Val-OMe HCl	A	2.2, DL-	84
13	Bz-Val	Val-OMe HCl	B	68, DL-	82
14	Bz-Val	Val-OMe HCl	A[j], CH_2Cl_2	1.4, DL-	76
15	Bz-Val	Val-OMe HCl	A[k], 30 min	9.1, DL-	81

[a]Procedure A, one-stage procedure: IBCF (10.7 mmol) was added, during a period of 60-90 min, to a mixture of R_1COOH (10.0 mmol), R_2NH_2 salt (10.7 mmol) and NMM (22.0 mmol) in DMF (5 mL) and THF (10 mL) cooled in an ice-bath. The mixture was stirred for another 15 min, warmed to 25 °C, diluted with water, extracted into EtOAc, and washed sequentially with 5% $NaHCO_3$, 10% citric acid, H_2O, and sat. NaCl. Drying and removal of solvent gave the crude peptide that was examined by HPLC for racemization. The product was isolated as a solid by triturating the crude peptide with hexane and filtration. [b]Procedure B, conventional two-stage procedure: to a solution of R_1COOH (10.0 mmol) and NMM (22.0 mol) in DMF (5 mL) and THF (10 mL) cooled in an ice-bath was added IBCF (10.7 mmol). The mixture was stirred for an additional 15 min. R_2NH_2 salt (10.7 mmol) was added and the mixture was stirred for 45 min. The mixture was warmed to 25 °C, diluted with water, extracted with EtOAc, and washed sequentially with 5% $NaHCO_3$, 10% citric acid, H_2O, and sat. NaCl. Drying and removal of solvent gave the crude peptide that was examined by HPLC for racemization. The product was isolated as a solid by triturating the crude peptide with hexane and filtration. [c]Enantiomeric purity was determined by HPLC analysis using a Daicel Chiralpak AS column (hexane:ethanol 4:1) for entries 1 to 4. [d]Diastereomeric purity was determined by HPLC analysis using a Metachem Inertsil ODS-2 column (0.05% phosphoric acid in CH_3CN:H_2O 1:1) for entries 5 to 15. [e]β-Alanine benzyl ester *p*-toluenesulfonate salt was used. [f]Procedure A was used except the reaction was carried out at 25 °C. [g]IBCF was added over a period of 5 hr. [h]Ref 1c. [i]Ref 3b. [j]Methylene chloride (25 mL) was used as the reaction solvent. [k]IBCF was added over a period of 30 min.

of racemization (2.2%, entry 12) is observed compared to the two-stage methodology (68%, entry 13). Shortening the addition time of IBCF from 90 to 30 min did increase the degree of racemization (entries 12 and 15). Slightly enhanced optical purity was found when methylene chloride was used as solvent (entries 12 and 14). In another example, the desired diastereomeric product was obtained exclusively (>99.9%) for the synthesis of a tripeptide (*Z*-Phe-Val-Ala-OMe)[9a] with 90% isolated yield (entry 9). For comparison with the conventional two-stage procedure (entry 10), the extent of racemization increased to 10%, which is 100 times more racemization than the one-stage procedure (entry 9). Joullié reported earlier that a dipeptide synthesis employing the conventional two-stage procedure in methylene chloride afforded *Z*-Ala-Val-OMe in 95% yield containing 28% of the DL-diastereomer.[3b] Once again, the undesired DL-diastereomer for this particular dipeptide was minimized to 0.1% utilizing the one-stage procedure (entry 7). This one-stage procedure also provided versatility and ease of operation for an industrial operation.[10]

The efficiency of this methodology for the synthesis of larger peptides was demonstrated by a [5+4] coupling of Boc-Tyr-Gly-Gly-Phe-Leu-OH (**5**) with H-Ala-Ala-Pro-Val-OMe (**6**). In spite of leucine's bulky substituent adjacent to the coupling site for pentapeptide **5**, nonapeptide **7** (Boc-Tyr-Gly-Gly-Phe-Leu-Ala-Ala-Pro-Val-OMe)[11] was obtained in 71% yield.

In conclusion, we have demonstrated a kinetically-controlled peptide synthesis, which is broad in scope, simple in operation, and advantageous in both chemical yield and optical purity. The most

noteworthy aspect of this methodology has been the retention of chiral integrity for hindered peptides, which should make it widely applicable for racemization-sensitive peptide coupling.

Acknowledgements

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4. Racemization occurs through either mixed anhydride or oxazolone intermediate, see: Kemp, D. S. in Ref. 1b, Chapter 7.
5. For symmetric anhydride formation, see: Bailey, P. D. In *An Introduction to Peptide Chemistry*; John Wiley & Sons: Chichester, New York, Brisbane, Toronto and Singapore, 1992; pp 131–132.
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8. An analytical sample of **1** was prepared by recrystallization from *t*-BuOH:heptane (2:1): mp 108–109°C; $[\alpha]_D^{25}$=5.5 (c=1, methanol); ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.26–7.60 (m, 14H), 6.50 (t, *J*=5.6 Hz, 1H), 5.20 (d, *J*=7.5 Hz, 1H), 5.02 (q, *J*=12.2 Hz, 2H), 4.37 (m, 1H), 3.50 (m, 2H), 3.05 (d, *J*=6.6 Hz, 2H), 2.45 (m, 2H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.9, 171.2, 155.3, 140.7, 139.8, 135.8, 135.6, 132.0, 129.8, 128.8, 128.6, 128.4, 128.2, 127.3, 127.0, 80.2, 66.5, 55.9, 38.5, 34.8, 33.9, 28.3. Anal. calcd for $C_{30}H_{34}N_2O_5$: C, 71.69; H, 6.82; N, 5.57. Found: C, 71.59; H, 6.80; N, 5.52. The enantiomeric purity was established by chiral HPLC analysis, see Table 1, footnote c. The chemical purity was determined by achiral HPLC using a Waters μBondapak C-18 column (methanol:aqueous PIC B5 buffer 4:1).
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10. A pilot-scale production of ca. 1,100 kilograms of **1** with chemical purity of >98% by HPLC[8] was successfully achieved employing this strategy.
11. Both peptides **5** and **6** were purchased from Bachem Bioscience Inc. and used as received. The hydrochloride salt of **6** was used in the coupling. Peptide **7**: $[\alpha]_D^{25}$=–66.8 (c=0.6, CH_3OH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.35–7.62 (m, 7H), 7.15–7.26 (m, 5H), 7.0 (d, *J*=8.4 Hz, 2H), 6.66 (d, *J*=8.4 Hz, 2H), 6.05 (d, *J*=8.1 Hz, 1H), 4.50–4.58 (m, 3H), 4.27–4.32 (m, 2H), 4.16–4.23 (m, 2H), 3.75 (t, *J*=5.2 Hz, 2H), 3.71 (t, *J*=5.3 Hz, 2H), 3.65 (s, 3H), 3.60–3.63 (m, 1H), 3.50–3.54 (m, 1H), 3.10 (dd, *J*=14.2, 5.2 Hz, 1H), 2.97 (dd, *J*=14.2, 5.2 Hz, 1H), 2.89 (dd, *J*=14.2, 8.3 Hz, 1H), 2.75 (m, 1H), 1.86–2.10 (m, 5H), 1.57–1.68 (m, 2H), 1.48–1.53 (m, 1H), 1.34 (s, 9H), 1.24 (dd, *J*=6.8, 1.3 Hz, 6H), 0.87–0.92 (m, 12H); thermospray-MS *m/z* (relative intensity) 1008 (M^+ +1) (6.2), 907 (M^+-Boc) (8.0), 688 (M^+-Boc-Tyr-Gly) (71.6), 631 (M^+-Boc-Tyr-Gly-Gly) (29.3), 484 (M^+-Boc-Tyr-Gly-Gly-Phe) (10), 371 (M^+-Boc-Tyr-Gly-Gly-Phe-Leu) (18.3); Anal. calcd for $C_{50}H_{73}N_9O_{13}$: C, 59.57; H, 7.30; N, 12.50. Found: C, 59.30; H, 7.16; N, 12.75.