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Pilot-Plant Preparation of an $\alpha_v\beta_3$ Integrin Antagonist: Process Development of a Carbonyldiimidazole Peptide Coupling

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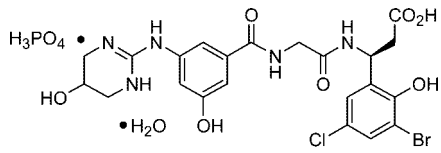
Abstract:

The process development of a peptide coupling with CDI is discussed. Various solvents, addition orders, stoichiometries, and reaction temperatures were investigated. A reliable crystallization procedure was also developed. The new process was piloted to provide 342 kg of product in two batches with an average 85% yield and 99% assay.

Introduction

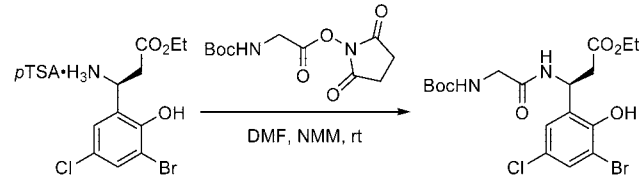
The integrin $\alpha_v\beta_3$ plays an essential role in angiogenesis, the process by which new blood vessels form from pre-existing blood vessels.[1] Angiogenesis is required for tumor growth, and therefore, antagonists of $\alpha_v\beta_3$ are being studied for the treatment of cancer. We have previously reported the synthesis of the left-hand tetrahydropyrimidine portion of antagonist **1** and a diastereoselective imino-Reformatsky reaction to install the (*S*)-β-amino acid functionality in the right-hand portion.[2] As part of a report describing the successful pilot-plant preparation of **1**, herein is detailed the chemical process research and development for installation of the glycine linker connecting the right- and left-hand portions of the molecule.[3] This paper describes improvements in the synthesis of **3**, a key intermediate in the synthesis of **1**.



1

Original Process. Initial supplies of **3** for preclinical development employed a process in which a DMF solution of **2** was stirred with *N*-methylmorpholine and *N*-*t*-Boc-glycine hydroxysuccinimide ester for 8 h at 20 °C. Vacuum distillation of the DMF, addition of ethyl acetate, a series of aqueous extractions, a second distillation for drying, back addition of ethyl acetate, and addition of heptane antisolvent provided **3** in 66% average yield. Our goals for improving the synthesis of **3** were (1) to lower the cost by replacing the expensive *N*-*t*-Boc-glycine hydroxysuccinimide ester, (2) to reduce the cycle time, and (3) to develop a more reliable process for the crystallization of **3**.

Scheme 1. **Original route used to prepare supplies of 3 for preclinical development**



2 **3**

Results and Discussion

The high cost of *N*-*t*-Boc-glycine hydroxysuccinimide ester ($315/mol) prompted us to investigate reactions of **2** with *N*-*t*-Boc-glycine ($51/mol) in the presence of several standard coupling agents. Carbonyldiimidazole (CDI) was chosen for several reasons: CDI is relatively inexpensive ($24/mol); coupling of amino acid salts with amines has been reported; the release of CO_2 provides a driving force for reaction; and the imidazole·*p*TSA byproduct could be easily removed by an aqueous extraction.[4]

Initial experiments were carried out at 25 °C by adding *N*-methylpyrrolidinone (NMP) to a 1:1 mixture of CDI and *N*-*t*-Boc-gylcine to generate the activated glycine derivative **4** as shown in Scheme 2.[5] Addition of 1.0 equiv of the solid amine

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(5) The addition of solvent to the two solids resulted in rapid reaction and release of CO_2. On small scale (1g) we did not experience any foaming or pressurization problems because we had sufficient headspace and a relatively large vent. Obviously, this mode of addition would need to be very carefully evaluated prior to scaling up.

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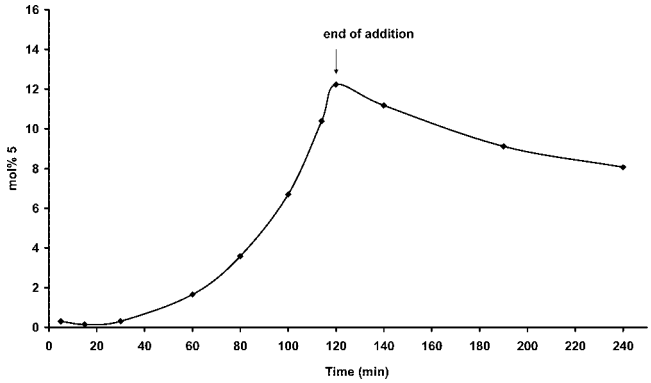
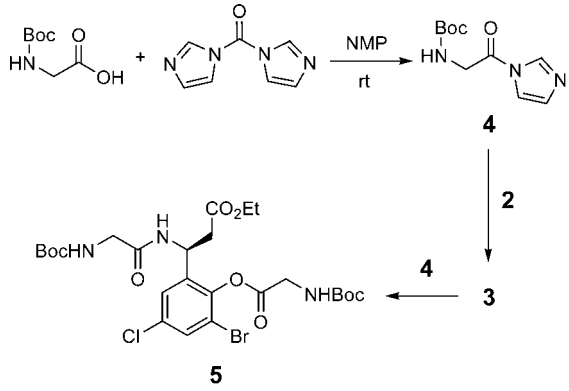


Figure 1. **Formation and subsequent consumption of 5 during a 2 h addition of 4 to 2.**

Scheme 2. **Preparation of 3 by the reaction of the imidazolide 4 with 2; *O*-acylation of 3 gives the diglycine impurity 5**



salt **2** to the solution of **4** provided **3** in 93% crude yield. The diglycine impurity **5**, derived from *O*-acylation of **3**, was also observed in 3 area %. Reaction monitoring of the homogeneous solution 30 min after the addition revealed a 7:2:1 ratio of **3**:**2**:**5**.[6] Aging of the reaction mixture for 4.5 h led to a 37:2:1 ratio of **3**:**2**:**5**. The observation that the ratio of **2** to **5** remains constant during the aging period suggests that **2** reacts with the phenolic glycine moiety of **5** to give 2 equiv of the desired product **3**.[7] These observations suggest that the rate-limiting step for complete conversion of **2** to **3** is the reaction of **2** with **5**.

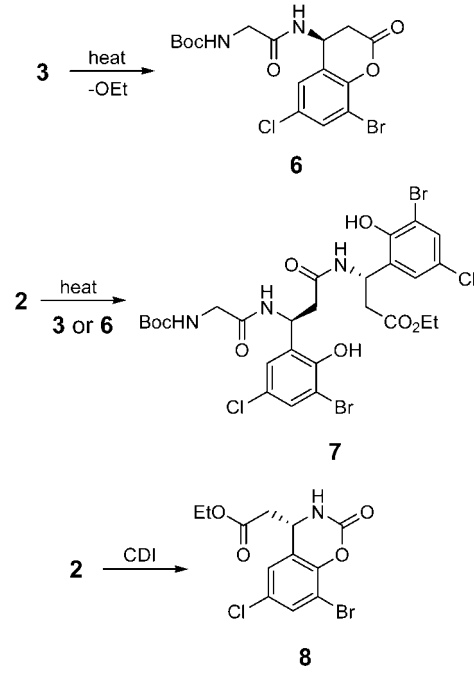
Having identified the feasibility of performing the desired conversion, process optimization studies were completed. Since the reaction of **2** with **5** to give **3** is relatively slow compared to the reaction of **2** with **4**, we wanted to design the process to minimize the reaction pathway involving **5**. We felt that this could be accomplished by reversing the addition order. As shown in Figure 1, **5** was formed in less than 1 mol % during the first 40 min of a 2 h addition of **4** to **2**. An exponential increase in **5** occurred as the concentration of **2** decreased and the concentration of **3** increased. Use of a large excess of the expensive starting material **2** was not a practical solution to overcome this concentration effect.

Reaction temperature and stoichiometry were identified as two critical process parameters affecting impurity formation and,

(6) We assume that **4** has been completely consumed at this point in the reaction. We did not develop an analytical method to confirm this assumption.

(7) An alternative mechanism is rate-limiting equilibration of **5** with imidazole to give **4** followed by reaction with **2** to give **3**.

Scheme 3. **Impurities formed during the synthesis of 3 using *N*-*t*-Boc-glycine and 1,1′-carbonyldiimidazole**



therefore, the reaction yield. For example, a reaction temperature of 60 °C gave significant amounts of the lactone **6** through intramolecular cyclization of **3** as shown in Scheme 3. Excess **2** combined with elevated reaction temperatures gave **7** through reaction of **2** with either **3** or **6**. Since significant amounts of **6** and **7** were observed within 90 min at 60 °C, we chose to run the reaction at 25 °C and accept the longer reaction time in exchange for lower impurity levels. Careful control of the stoichiometry was necessary to minimize impurites. Excess CDI led to the formation of the carbamate **8** by intramolecular cyclization of **2**.[8] Even though excess **4** leads to **5**, in practice, a nominal excess of **4** was necessary for high conversion of the expensive **2**.[9] Small amounts of unreacted **2** were easily removed by an acidic extraction.

Our next set of experiments focused on replacing NMP as the reaction solvent. Ethyl acetate, tetrahydrofuran, and toluene were screened as alternative solvents. In each case the solvent was added to a 1:1 mixture of CDI and *N*-*t*-Boc-glycine followed by the addition of 1.0 equiv of **2**.[5] The heterogeneous reactions were stirred at room temperature for 5 h and monitored by HPLC. A significant increase in the amount of **7** was observed when toluene was employed as the solvent. The reaction rates and impurity profiles with ethyl acetate or THF as solvent were essentially identical to those observed with NMP. Therefore, replacement of NMP with ethyl acetate or THF appeared promising, but one more issue had to be addressed. For ease of reaction screening in the laboratory, we had been adding solid **2** to a solution of **4**, but addition of the agglomerated solid was not practical for our pilot plant. Therefore, a process employing a slurry or a solution of **2** was needed. Attempts to slurry **2** in ethyl acetate or THF were not

(8) The urea resulting from addition of 2 equiv of **2** to CDI was not observed under these reaction conditions.

(9) Due to time constraints, we did not develop an assay for CDI or **4**. All bulk materials were use-tested in the lab prior to the scale-up.

successful because the solids stuck together to form a solid mass that was not readily stirred. Therefore, we could not completely replace NMP because it was needed to dissolve **2**, but we were able to significantly reduce the amount by preparing **4** in ethyl acetate.[10]

Having defined the method of interaction of the substrate with the reagents, we now focused our studies on the transfer of the technology to a two-reactor workstation in the pilot plant. This was successfully accomplished employing the following protocol. First, a solution of **4** in ethyl acetate was prepared by adding a solution of *N*-*t*-Boc-glycine from reactor 1 to a slurry of CDI in reactor 2. Reactor 1 was rinsed with ethyl acetate, and a solution of **2** in NMP was prepared in it. The ethyl acetate solution of **4** in reactor 2 was quickly added to the solution of **2** in reactor 1, and the heterogeneous mixture was stirred at room temperature for 3.5 h.[11] The reaction rates and impurity profiles with the mixed solvent system using inverse addition were essentially identical to those observed with NMP in the normal addition mode. The reaction was diluted with ethyl acetate, washed with 1 M HCl, and washed with water to provide an ethyl acetate solution of **3**.

Our third goal was to improve the crystallization of **3** from the ethyl acetate solution. The original process called for a vacuum distillation of the ethyl acetate solution to remove water. Ethyl acetate was back added, and the solution was warmed to 55 °C. Crystallization of the product was achieved by addition of heptane and seed crystals, and cooling to 0 °C. The procedure was unreliable, and oiling of the product was periodically observed after the heptane charge or during the cool down. Substitution of heptane with hexane helped alleviate the oiling problem, but we wanted to avoid handling hexane on large scale. Because the solubility of **3** was very low in heptane and very high in ethyl acetate, **3** had a propensity to become highly supersaturated and oil out during crystallization attempts from a two-solvent system of heptane/ethyl acetate. It appeared that an alternative antisolvent was needed to provide a bridge from high solubility to low solubility and enable a controlled crystallization of **3**. Since **3** had intermediate solubility in toluene, it was a logical choice, but on the basis of solubility data, it became clear that a toluene/ethyl acetate system would result in low yields and heptane was necessary for recovery. Therefore, we decided to try a three-solvent system for the crystallization of **3**. Solubility data at 55, 25, and −5 °C for **3** in various ratios of ethyl acetate, toluene, and heptane are shown in Table 1.

Response surfaces were generated, and from these data we determined that an end point consisting of 7 volumes of a 1:1:3 ethyl acetate:toluene:heptane mixture would allow for maximum recovery of **3** and good operability in a pilot plant. We found that decreasing the relative amount of ethyl acetate led to oiling out of the product. The desired ratio was achieved through an atmospheric distillation of ethyl acetate followed by addition of toluene at 55 °C. Quantitation of ethyl acetate was achieved by means of GC analysis with toluene providing an internal standard. Ethyl acetate could be back added if too much was removed by distillation, or toluene could be added to obtain the desired 1:1 ratio in the case of under-distillation. Heptane was added slowly during three charges because of the slow desaturation rate of **3**. The first heptane charge was designed to give 20% supersaturation of **3** since oiling had been observed at 40% supersaturation. Seeding, two additional heptane charges, and controlled slow cooling to −5 °C resulted in consistent crystallization of the product.

Table 1. **Solubility of 3 in various mixtures of EtOAc, toluene, and heptane at 55, 25, and −5 °C**

solvent composition (vol %)			solubility of **3** (mg/mL)		
EtOAc	toluene	heptane	55 °C	25 °C	−5 °C
20	20	60	50.9	20.2	8.1
30	10	60	81.5	20.7	13.5
10	30	60	22.4	10.3	3.7
10	10	80	7.0	3.0	2.1
26.5	26.5	47	116	30.2	13.8
33.3	33.3	33.4	168	63.0	22.2
100	0	0	380	244	166
0	100	0	141	95.0	12.1
0	0	100	0.18	0.05	0.01
50	0	50	193	117	35.3
0	50	50	10.8	7.67	2.15
50	50	0	340	167	101

Table 2. **Results from kilo lab and pilot-plant preparations of 3 from reaction of 2 with 4 using an optimized process**

2 (kg)	**4** (equiv)	**3** (% yield)	**3** (% assay)	**3** (mol % in mother liquor)
2.75	1.05	74.5	98.7	11.4
203	1.10	84.8	98.9	7.1
203	1.13	86.4	99.7	8.0

The new process was run once in a kilo laboratory and twice in a pilot plant. The data are summarized in Table 2. The initial scale up to 2.75 kg of **2**, and 1.05 equiv of **4** provided **3** in 74.5% yield with a 98.7% assay. During the crystallization portion of the process the ethyl acetate was under-distilled and, therefore, additional toluene and heptane were used. This resulted in an 11 mol % loss of product to the mother liquor. The first 203 kg batch of **2** run in the pilot plant used 1.10 equiv of **4** based on laboratory use tests. The desired product **3** was obtained in 84.8% yield with an assay of 98.9%. We increased **4** in the second batch to 1.13 equiv to increase the conversion of **2** to **3**. The product was obtained in 86.4% yield with an assay of 99.7%. Since overdistillation of ethyl acetate was achieved during the crystallization in both pilot plant batches, ethyl acetate was back added and product loss to the mother liquor was 7.1 and 8.0 mol%. We attribute the lower mass balance of the kilo laboratory preparation of **3** to the lower purity **2** used in that synthesis.[12]

In summary, a procedure was developed and demonstrated on a pilot-plant scale that *N*-*t*-Boc-glycine in the presence of CDI is a cost-effective replacement for *N*-*t*-Boc-glycine hydroxysuccinimide ester for the synthesis of **3**. Coupled with improvements in the crystallization procedure, the new process provided 342 kg of product in two batches with an average 85% yield and 99% assay.

(10) We chose ethyl acetate over THF because of the cost difference.
(11) Slow addition of **4** to **2** resulted in longer reaction times due to the slow build up of **5** and subsequent slow reaction of **5** with **2** to give **3**.
(12) The purity of **2** was 83.1, 88.1, and 91.6% in the kilo lab prep and two piloting preps, respectively.

Experimental Section

(3*S*)-*N*-[(1,1-Dimethylethoxy)carbonyl]glycyl-3-[3-bromo-5-chloro-2-hydroxyphenyl]-*β*-alanine ethyl ester (3). A solution of *N*-*t*-Boc-glycine (81.2 kg, 464 mol) at 25 °C in ethyl acetate (428 L) in reactor 1 was transferred to a slurry of CDI (75.2 kg, 464 mol) at 25 °C in ethyl acetate (325 L) in reactor 2. Reactor 1 was rinsed with ethyl acetate (50 L), and the rinse was transferred to reactor 2. Minor foaming was observed, but the pressure did not increase. The clear yellow solution was stirred for 21 h at 25 °C. Note: A reaction time of only 1 h is necessary for complete conversion to **4**. A solution of **2** (221.6 kg, 91.6% assay, 410.3 mol) in NMP (239 L) was prepared in reactor 1 at 25 °C. The solution of **4** in reactor 2 was transferred to the solution of **2** in reactor 1 over 19 min. Reactor 2 was rinsed with ethyl acetate (50 L), and the rinse was transferred to reactor 1. The reaction mixture was sampled after 4 h at 25 °C, and HPLC analysis showed 2.7 area % of **2** remained. The reaction mixture was diluted with ethyl acetate (828 L), washed once with 1 M HCl (aq) (1188 L), and washed three times with water (3 × 1170 L). The organic layer was concentrated by atmospheric distillation (71−84 °C pot temperature) of ethyl acetate (1090 L) over a 7 h period, and then the solution was cooled to 55 °C. Toluene (275 L) was charged at a rate to keep the solution above 50 °C. The solution was sampled, and GC analysis showed that the weight ratio of ethyl acetate:toluene was 0.40. Ethyl acetate (169 L) was added to achieve the desired 1.03 weight ratio. Heptane (550 L) was charged at a rate to keep the solution above 45 °C, and the solution was reheated to 50 °C. Seeds of **3** (1.7 kg) were added, and the slurry was stirred at 50 °C for 4 h. Heptane (137 L) was charged and the slurry was stirred for 100 min at 50 °C. Heptane (137 L) was added and the slurry was cooled to 45 °C over 50 min and held for 75 min. The slurry was cooled to 30 °C over 3.25 h and held for 1 h, and then cooled to −5 °C over 3.42 h and held for 37.25 h. The slurry was filtered, washed with a mixture of cold toluene/heptane (217 L/412 L), and dried in a tumble dryer to give **3** (170.6 kg, 84.8% yield adjusted for seeds and assay). The product was 98.9 wt % pure by HPLC analysis against a standard. HPLC method: YMC basic 150 mm × 4.6 mm, 5 μm, 25 °C, 1.0 mL/min, 220 nm, 50% pH 7 buffer: 50% MeOH 20 min gradient up to 80% MeOH, retention times (min), **2**(7.48), **3** (10.98), **5** (12.29), **6** (8.89), **7** (11.97), **8** (8.29). Characterization for a typical standard of **3** follows: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (t, J = 7.5, 3H), 1.43 (s, 9H), 2.81−2.95 (m, 2H), 3.75−3.87 (m, 2H), 4.13 (q, J = 7.5, 2H), 5.09−5.17 (m, 1H), 5.53−5.98 (m, 1H), 7.13 (d, J = 3.0 Hz, 1H), 7.22−7.35 (br s, 1H), 7.41 (d, J = 3.0 Hz, 1H), 7.60 (d, J = 10 Hz, 1H). ^{13}CNMR ($CDCl_3$, 125 MHz) 14.0, 28.2, 38.0, 44.3, 46.1, 61.0, 80.5, 111.9, 125.5, 127.0, 129.2, 131.2, 149.4, 156.1, 170.0, 170.7.

(3*S*)-*N*-[(1,1-Dimethylethoxy)carbonyl]glycyl-3-[3-bromo-5-chloro-2-(((1,1-dimethylethoxy)carbonyl)glycyl)oxy]-*β*-alanine ethyl ester (5). *N*-methylpyrrolidinone (10 mL) was added to a flask containing *N*-*t*-Boc-glycine (4.43 g, 25.3 mmol) and 1,1′-carbonyldiimidazole (4.10 g, 25.3 mmol), and the solution was stirred for 1 h. **2** (5.00 g, 10.1 mmol) was added, and the solution was stirred for 3 h. Reaction monitoring revealed a 1.3:1 ratio of **3**:**5**. The addition of *N*-methylmorpholine (0.71 mL, 6.4 mmol) did not significantly change the product ratio after stirring for 15 h. In a separate flask *N*-methylpyrrolidinone (8 mL) was added to *N*-*t*-Boc-glycine (2.66 g, 15.2 mmol) and carbonyldiimidazole (2.46 g, 15.2 mmol), and the solution was stirred for 1.25 h. The reaction mixture containing **3** and **5** was added to the solution of **4** and stirred for 1 h. The ratio of **3**:**5** did not significantly change. Ethyl acetate (50 mL) was added, and the organic was washed with brine (50 mL), 1 M HCl (2 × 50 mL), sat. $NaHCO_3$ (50 mL), dried over $NaSO_4$, and concentrated to give a mixture of **3**, **5**, and NMP (4.93 g) as a yellow foam. The foam was dissolved in 50% ethylacetate/heptane (15 mL), washed with water (15 mL), dried over $NaSO_4$, and concentrated. Separation of **3** and **5** was achieved by reverse phase chromatography using 50% ACN in water to provide 1.98 g (30.8% yield) of analytically pure **5**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (t, J = 7.5, 3H), 1.44 (s, 9H), 1.47 (s, 9H), 1.73 (s, 1H), 2.84 (br d, J = 7.5, 2H), 3.75 (br d, J = 7.5, 2H), 4.11 (q, J = 7.5, 2H), 4.22 (br d, J = 5.0, 2H), 5.22−5.32 (br s, 1H), 5.38−5.47 (m, 2H), 7.36 (d, J = 3.5, 1H), 7.52 (d, J = 3.5, 1H). Anal. Calcd for $C_{25}H_{35}BrClN_3O_9$: C, 47.14; H, 5.54; Br, 12.55; Cl, 5.57; N, 6.60. Found: C, 46.74; H, 5.54; Br, 12.03; Cl, 5.86; N, 6.49.

(*S*)-Ethyl 2-(8-bromo-6-chloro-2-oxo-3,4-dihydro-2H-benzo[*e*][1,3]oxazin-4-yl)acetate (8). *N*-methylpyrrolidinone (5 mL) was added to a flask containing **2** (2.00 g, 4.04 mmol) and 1,1′-carbonyldiimidazole (0.3277 g, 2.02 mmol), and the solution was stirred for 7 h. Triethylamine (0.56 mL, 4.02 mmol) was added, and the solution was stirred overnight. Note: We were initially attempting to make the urea resulting from addition of 2 equiv of **2** to CDI, but none was observed under these reaction conditions. A second charge of 1,1′-carbonyldiimidazole (0.3277 g, 2.02 mmol) was added and the solution was stirred overnight. Ethyl acetate (20 mL) was added and the organic was washed with brine (20 mL), 1 M HCl (20 mL), sat. $NaHCO_3$ (20 mL), dried over $NaSO_4$, and concentrated to give 1.06 g (75% yield) of **8** as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, 3H), 2.78 (d, 2H), 4.22 (q, 2H), 4.94 (td, 1H), 6.27 (br d, 1H), 7.07 (d, 1H), 7.56 (d, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz)14.1, 43.0, 49.9, 61.7, 111.5, 122.0, 124.6, 130.0, 132.9, 145.5, 148.7, 170.2. Anal. Calcd for $C_{12}H_{11}BrClNO_4$: C, 41.35; H, 3.18; Br, 22.92; Cl, 10.17; N, 4.02. Found: C, 41.50; H, 3.23; Br, 24.19; Cl, 9.56; N, 4.09.

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