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Synthesis of ABT-378, an HIV Protease Inhibitor Candidate: Avoiding the Use of Carbodiimides in a Difficult Peptide Coupling

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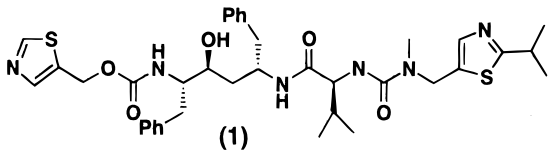
Abstract:

An alternative to carbodiimide-mediated peptide coupling protocols has been developed for a carboxylic acid prone to decomposition by polymerization. This method, involving the in situ generation of an acyl imidazolide, has been applied to the preparation of a lead clinical HIV protease inhibitor candidate, ABT-378. The nature of the polymerization and optimization of the new reaction conditions are presented.

The approval of the first HIV protease inhibitors in early 1996 provided the world with powerful new weapons in the fight against HIV, the virus responsible for AIDS.[1] HIV protease is an enzyme critical to the life cycle of the virus, and its inhibition disrupts viral replication, resulting in the formation of immature, *noninfectious* viral particles.[2] While the protease inhibitors, combined together and in "drug cocktails" with various reverse transcriptase inhibitors, can be extremely potent in reducing blood levels of HIV, the clinical benefit observed eventually degrades due to the development of drug resistance, arising from predictable mutations in the virus.[3] As a result, research continues toward the development of new and more powerful protease inhibitors.[4] Among this first generation of effective HIV protease inhibitors is Abbott Laboratories' Ritonavir **(1)**[5] (Norvir). Abbott's next generation candidate, ABT-378 **(2)**, is showing considerable promise as well.[6]



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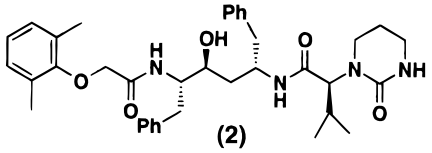
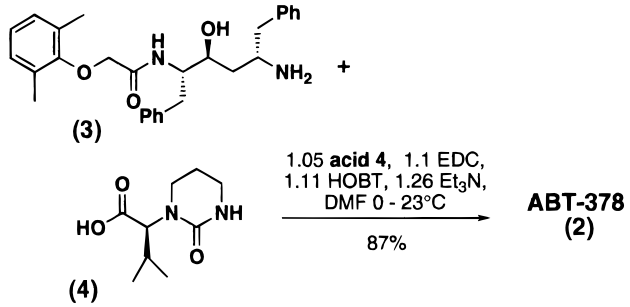


(2)

The development of ABT-378 **(2)** has been justifiably rapid, and, as might be expected, numerous difficulties were encountered in making the fast-paced transition from discovery synthesis to a scale suitable for the preparation of multikilogram quantities of drug. One transformation in the synthesis which presented considerable complication is the peptide coupling shown below, in which amine **3**[7] and L-valine-derived acid **4**[8] are coupled, affording ABT-378 **(2)**. Being the final step in the synthetic sequence, this chemistry needed to be high yielding and efficient and to produce **2** in high purity with minimal racemization.



The original discovery synthesis utilized 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDC) activation of **4** to prepare the *N*-hydroxybenzotriazole (HOBT) ester. While the HOBT ester of **4** was an efficient coupling partner, leading to clean formation of **2**, these two reagents posed numerous problems for larger scale work. The most prominent among these deterrent factors were the toxicity, high cost, and limited availability of EDC.[9] Therefore, we set out to determine a more suitable method to perform this coupling. Our goal was to develop a process as high yielding and efficient as the EDC/HOBT process with none of the hazards and excessive costs associated with those reagents.

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(6) Sham, H. L.; Norbeck, D. W.; Chen, X.; Betebenner, D. A.; Kempf, D. J.; Herrin, T. R.; Kumar, G. N.; Condon, S. L.; Cooper, A. J.; Dickman, D. A.; Hannick, S. M.; Kolaczkowski, L.; Oliver, P. A.; Plata, D. J.; Stengel, P. J.; Stoner, E. J.; Tien, J.-H. J.; Liu, J.-H.; Patel, K. M. U.S. Patent Appl. 95-57226; *Chem. Abstr.* **1997**, *127*, 122001.

(7) Prepared from diamino alcohol intermediate used in Ritonavir synthesis, see: (a) Stuk, T. L.; Haight, A. R.; Scarpetti, D.; Allen, M. S.; Menzia, J. A.; Robbins, T. A.; Parekh, S. I.; Langridge, D. C.; Tien, J.-H. J.; Pariza, R. J.; Kerdesky, F. A. J. *J. Org. Chem.* **1994**, *59*, 4040. (b) Haight, A. R.; Stuk, T. L.; Menzia, J. A.; Robbins, T. A. *Tetrahedron Lett.* **1997**, *38* (24), 4191 and references therein.

(8) Prepared from phenoxycarbonyl-L-valine and chloropropylamine hydrochloride, see: Stengel, P. J.; Cooper, A. J.; Oliver, P. A.; Patel, K. M., manuscript in preparation.

(9) For an excellent review of this reagent, see: *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: New York, 1995; Vol. 4, p 2430.

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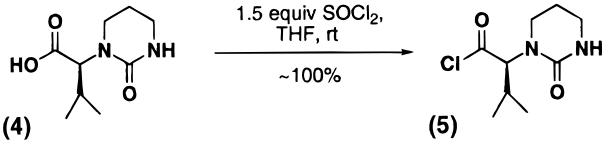
Our initial efforts involved the screening of common peptide coupling methods. Of those examined, couplings with propyl phosphonic anhydride[10] in the presence of *N*-methylmorpholine proved the most viable. Unfortunately, this methodology suffered by comparison to the original process in two respects. First, propyl phosphonic anhydride/*N*-methylmorpholine failed to give full conversion of amine **3** to **2**, and second, these reagents appeared to cause some degradation of **3**, which made the isolation and purification of **2** more difficult.

The extent of conversion of amine **3** to product **2** was a critical factor in the development of this step of the synthesis. We found that levels of unreacted amine **3** above a few percent made the final purification of **2** by crystallization exceedingly difficult, and substantial losses in yield were observed in efforts to achieve the purities needed for clinical use. Therefore, a very high conversion of amine **3** to inhibitor candidate **2** was considered an essential feature of any workable coupling protocol.

Our subsequent efforts were hampered by our inability to prepare active esters of **4** without using carbodiimides with their attendant waste and toxicity. Treatment of **4** with oxalyl chloride, acyl chlorides, and numerous chloroformates, followed by reaction with *N*-hydroxysuccinimide, *N*-hydroxybenzotriazole, and the like, failed to yield the desired activated esters in any appreciable quantity. In these experiments, no unreacted acid remained, having been converted into an intractable semisolid mass. It became clear that the activation of **4** achieved with oxalyl chloride and other reagents generated an intermediate species so unstable that it degraded nearly instantaneously (even at low temperatures). This made the subsequent discovery that acyl chloride **5** could be prepared with $SOCl_2$ or $POCl_3$ and then isolated as a stable crystalline solid all the more surprising.[11] It was this discovery that led us to evaluate the direct coupling of acyl chloride **5** with amine **3** as the most desirable approach. The fact that acyl chloride **5** could be isolated provided evidence that it was not responsible for the degradation observed earlier.



(4) (5)

With a convenient synthesis of **5** in hand, many activated esters of **4** were now accessible to us; however, these compounds remained less desirable than any direct coupling protocol. Our initial attempts to couple acyl chloride **5** with amine **3** in the presence of pyridine in a variety of solvents were promising. The reactions were clean but failed to produce complete reaction of **3** to **2**, with conversions on the order of 15−60% being common. While the conversion ratio (and therefore the yield) could be improved by using larger excesses of pyridine, it was quickly apparent that we would never achieve our desired goal of complete conversion with this base. We were resistant to improving the conversion ratio by further increasing the amount of acyl chloride **5** used, due to the fact that acid **4** represented a significant contributor to the overall cost of the synthesis. Although we had examined various methods for the removal of unreacted **3** with aqueous washes, these proved unreliable and problematic.

We screened a large array of amine and inorganic bases in this reaction, including pyridine, *N*,*N*-dimethylaminopyridine, Na_2CO_3, $NaHCO_3$, *N*-methylmorpholine, and triethylamine, but failed to find a suitable set of reaction conditions. Reaction of **5** with imidazole produced the expected acyl imidazolide, which we found to be superior to anything we had tried. We were also pleased to find that the addition of **5** to amine **3** and imidazole produced even higher conversions and cleaner reactions. Subsequently, a "first generation" optimized procedure was developed which involved the addition of a DMF solution of 1.1−1.3 equiv of acyl chloride **5** to a mixture of 1.0 equiv of amine **3** and 1.3−1.5 equiv of imidazole in EtOAc, producing **2** in very high purities, with conversions of greater than 95%. This process proved useful enough to prepare multikilogram quantities of **2**.

While pleased with our working process, we remained committed to further improvement, especially in light of a second pivotal discovery. We had initially assumed that a slight excess of **5** was needed to cover losses due to hydrolysis which had occurred during processing. The byproduct of this hydrolysis, acid **4**, was probably being removed during the aqueous workup. During the quench of these coupling reactions, a fine solid appeared at the interface of the aqueous and organic layers. In larger scale reactions, this solid remained in suspension and made further processing (layer separations, etc.) extremely difficult. Filtration to remove this solid was possible but problematic owing to the extremely small particle size, which slowed processing and hampered throughput.[12]

To our surprise, isolation and characterization of this material revealed it to be a polymer comprised of acid **4** monomer units. Based on our analytical data, we believe **6** best represents the structure of this polymer, although we cannot completely eliminate structure **7** or structures consisting of both types of polymer linkages. The "head-to-tail" orientation of polymer **6** is supported principally by the IR spectrum, which lacks the presence of ester carbonyl stretches, instead showing a single large carbonyl absorption at 1678 cm^{-1}, and the ^{13}C NMR spectrum, which shows two major carbonyl peaks at 173.1 and 157.6 ppm in $CDCl_3$.[13]

As expected, the range of polymer molecular weights varies with experimental parameters. Polymers isolated from a number of laboratory and early pilot plant runs have molecular weight distributions varying from around 800 to over 4000 amu. The most common molecular weights correspond to 14−16 monomer units. Mass spectral data and

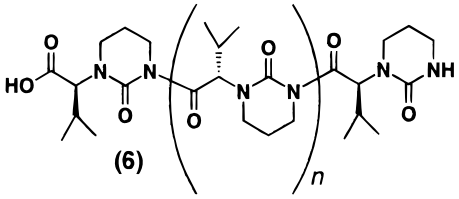
(10) For an excellent review of this reagent, see: *Encyclopedia of Reagents for Organic Synthesis*; Paquette, L. A., Ed.; Wiley: New York, 1995; Vol. 6, p 4336.

(11) Xi, N.; Ciufolini, M. A. *Tetrahedron Lett.* **1995**, *36* (17), 6595.

(12) Crude reaction mixtures containing large amounts of this polymer have the consistency of latex paint.

(13) We have assigned these two peaks as the amide carbonyl and the urea carbonyl, respectively.

elemental analysis further suggest that only acid **4** units are incorporated in the polymer, as shown. Hydrolysis of these polymers with strong base regenerates acid **4**.



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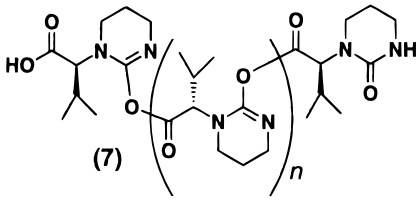


(7)

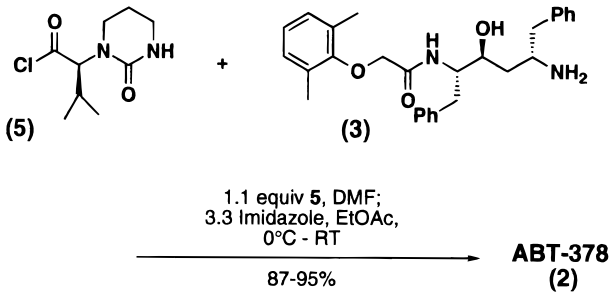
The formation of this polymer was, in our minds, another indication of the instability of certain activated derivatives of **4**. While we have not completely determined the cause of this polymerization, we were fortunate in that the cure was quickly discovered. When 3 equiv or more of imidazole was used, the polymerization was completely prevented. The added benefits of this simple and inexpensive change are numerous and include easier processing, nearly quantitative conversion of **3** to **2** (typically greater than 99%), cleaner crude reaction mixtures, and higher yields. While the reactions become slower as more imidazole is used, it is an acceptable cost relative to the benefits achieved.

Discussion and Final Optimization

The polymerization of acyl chloride **5** is intriguing. At lower imidazole concentrations (<1 equiv), polymerization is the main reaction pathway of **5**, while when a sufficient excess of imidazole is used, it is a virtually nonexistant process. Our analysis of this phenonmenon is based on the dual nature of imidazole as base and nucleophile. We know from experience that the coupling of acyl chlorides with amines is a fast process, producing HCl at a correspondingly fast rate. If this acid is not removed quickly enough, it is our belief that HCl-activated **5** becomes prone to polymerization and a competition is set up between coupling, removal of acid by imidazole, and polymerization. As expected, couplings with less than 2 equiv of imidazole are extremely rapid.

When, however, more than 3 equiv of imidazole is employed in a coupling reaction, there is sufficient base present to compensate for any initial coupling and HCl generation which take place. Additionally, imidazole now becomes a competitive nucleophile, allowing for the formation of the acyl imidazolide derivative of **4**, which couples with **3** relatively slowly and is quite stable. We have observed an experimental "gray area" which occurs when imidazole is present at roughly 1.7−3.0 equiv. Experimentally, this region produces results intermediate to the two extremes, with yields varying with other experimental parameters such as temperature and agitation.

We subsequently screened a large number of other bases, including 1,2,4-triazole, thiazole, and various commercially



(5) (3)

available alkylated and nitrated imidazole derivatives, finding them to be inferior to imidazole with respect to this reaction.

Conclusion

Presently we have achieved a coupling protocol which is suitable for bulk drug production. Our non-carbodiimide-mediated coupling of polymerization-prone carboxylic acid **4** and amine **3** leading to ABT-378 is efficient and inexpensive and relies on readily available raw materials. We have avoided a number of safety and environmental issues by the substitution of thionyl chloride and imidazole for EDC and HOBT. Furthermore, while we have a viable, reproducible, large scale process, work continues to further refine the chemistry. This process has been run on a multikilogram scale numerous times, and a version of this protocol involving a different amine partner has been run successfully on over a 200-kg scale.

Experimental Section

All reactions were performed under a positive pressure of nitrogen. Solvent concentration was accomplished with a Büchi rotary evaporator at ca. 15 mmHg pressure using a vacuum pump (*not* water aspiration).[14] Commercial grade anhydrous solvents and reagents were used without further purification. HPLC purities were determined by peak area percent.

Preparation of Acyl Chloride 5. Acid **4** (25.8 g, 0.129 mol, 1.1 equiv) and 590 mL of THF were charged to a 1-L, three-necked, round-bottomed flask and cooled to 0 °C. Neat thionyl chloride (17.4 g, 10.7 mL, 1.25 equiv) was added dropwise over 15 min via a pressure-equalizing addition funnel. When the addition was complete, the reaction mixture was warmed to room temperature and stirred for an additional 1 h. HPLC analysis of samples prepared by quenching into MeOH showed no unreacted **4** at this time. The reaction mixture was then evaporated to dryness in vacuo and 150 mL of heptane added to facilitate the removal of unreacted thionyl chloride azeotropically. The heptane slurry was evaporated to dryness in vacuo, affording acyl chloride **5** as a hygroscopic off-white solid (HPLC analysis, >98% pure). The crude **5** was immediately slurried in 180 mL of anhydrous DMF and held for use in the next step.[15]

Acyl Chloride 5. ^{1}H NMR (300 MHz, CD_3CN): δ 12.5 (v br s, 1H), 4.61 (d, J = 3 Hz, 1H), 3.61 (app t, 4H), 2.44

(14) Water aspiration can lead to partial hydrolysis of acyl chloride **5** during reduction in volume.

(15) Acyl chloride **5** slowly degrades in DMF solution at room temperature and should be used within 2−3 h for best results; solid **5** is stable under nitrogen indefinitely.

(d sept, $J = 3$, 6.5 Hz, 1H), 2.16 (m, 2H), 1.16 (d, $J = 6.5$ Hz, 3H), 1.01 (d, $J = 6.5$ Hz, 3H). ^{13}C NMR (75 MHz, CD_3CN): δ 165.2, 156.0, 65.5, 41.9, 39.0, 29.0, 19.0, 16.7, 16.4. MS (DCI, NH_3) 215 $(M + H)^+$.

Coupling Procedure: Synthesis of ABT-378, 2. A dry 2-L, three-necked, jacketed, round-bottomed flask equipped with a mechanical stirrer, nitrogen inlet adapter, and pressure-equalizing addition funnel was charged with amine **3** (52.4 g, 0.117 mol, 1.0 equiv), imidazole (26.3 g, 3.3 equiv), and 590 mL of ethyl acetate, and the contents were cooled to 0 °C. To this reaction mixture was slowly added the DMF slurry of acyl chloride **5** at such a rate as to maintain the internal temperature below 5 °C (about 30 min).[16] The reaction mixture was stirred for an additional 30 min at 0 °C and then warmed to room temperature overnight. HPLC analysis revealed complete consumption of starting material (amine **3**) after 12 h.[17]

The reaction mixture was quenched by the addition of 0.2 N aqueous HCl (1 L) and 100 mL of ethyl acetate and allowed to stir for 30 min. The layers were separated,[18] and the organic layer was washed once with 5% (w/w) aqueous $NaHCO_3$ (500 mL) and twice with 500 mL of distilled water. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo, affording crude **2** as a cream-colored solid, 71.5 g (97%, HPLC purity >98%, although DMF and ethyl acetate are present).

The solid was dissolved in 350 mL of ethyl acetate at 70 °C, and 350 mL of heptane was slowly added.[19] After being reheated to 70 °C, the solution was slowly cooled to 10 °C. The product was collected by filtration; the wet product cake was washed with 100 mL of 0 °C 1:1 (v/v) heptane/ethyl acetate. The solid was dried in vacuo at 60 °C for 24 h, affording 63.65 g (86.2%) of product as a colorless solid. A second crop of off-white crystals was isolated from the mother liquors, 4.30 g (5.8%), for a total of 67.95 g (92% yield). Both crops of product **2** assayed as >99% pure by HPLC.

Characterization Data for ABT-378, 2. Mp (EtOAc): 124−127 °C (uncorrected).[20] IR (KBr): 3413, 3335, 3289, 3060, 2966, 1671, 1650, 1624, 1545, 1520, 1453, 1189, 701 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30−7.13 (m, 10H), 7.02−6.92 (m, 3H), 6.86 (v br s, 1H), 5.68 (br s, 1H), 4.25 (m, 1H), 4.19 (app d, $J = 10$ Hz, 2H), 4.19 (m, 2H), 3.78 (m, app d sept, 1H), 3.12 (m, 1H), 3.06 (m, 2H), 2.97 (d, $J = 7.6$ Hz, 2H), 2.88 (m, 1H), 2.81 (app ABX dd, $J = 14$, 5.2 Hz, 1H), 2.68 (app ABX, dd, $J = 14$, 9.5 Hz, 1H), 2.23 (m, 1H), 2.18 (s, 6H), 1.83 (s, 1H), 1.74 (m, 2H), 1.53 (m, 1H), 1.28 (m, 2H), 0.83 (app t, $J = 7$ Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7, 168.8, 156.5, 154.2, 138.1, 138.0, 130.3, 129,3, 129.2, 129.0, 128.4, 128.2, 126.3, 126.0, 124.6, 70.2, 69.7, 63.1, 54.4, 48.7, 41.8, 41.1, 40.8, 40.0, 38.2, 25.4, 21.7, 19.6, 18.7, 16.1. MS (ESI): 629 $(M + H)^+$, 651 $(M + Na)^+$. Anal. Calcd for $C_{37}H_{48}N_4O_5$: C, 70.66; H, 7.69; N 8.91. Found: C, 70.26; H, 7.73; N, 8.79.

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(16) Even with very efficient cooling, this rate of addition is recommended to avoid polymer formation and to ensure complete conversion of amine **3** to product.
(17) Typical reaction times vary from 12 to 24 h.
(18) If polymer is formed, it will appear as a finely divided solid in suspension. It can be removed at this stage by filtration through diatomaceous earth.
(19) The recovery of product is greatly dependent upon the water content of the recrystallization solution. A water content of this solution above 0.2% will result in a significant loss of product.
(20) Product crystallized in this manner contains approximately 2% residual ethyl acetate which cannot be removed by further drying. Removal of this residual solvent requires additional processing.