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A Convenient and Scalable Synthesis of Ethyl *N*-[(2-Boc-amino)ethyl]glycinate and Its Hydrochloride. Key Intermediates for Peptide Nucleic Acid Synthesis

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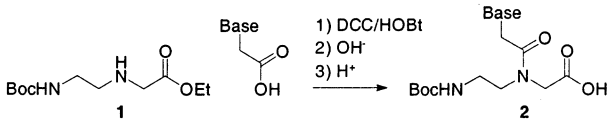
Abstract: An improved synthesis of ethyl *N*-[(2-Boc-amino)-ethyl]glycinate and its hydrochloride salt is reported. The synthesis is based on the reductive alkylation of Boc-ethylenediamine with ethyl glyoxylate hydrate and furnishes the title compound in near quantitative yield and high purity without chromatography. This compound is suitable, as is, for the synthesis peptide nucleic acid monomers. Further, conversion to the hydrochloride salt provides a stable, nonhygroscopic solid that is a convenient form for handling and storage.

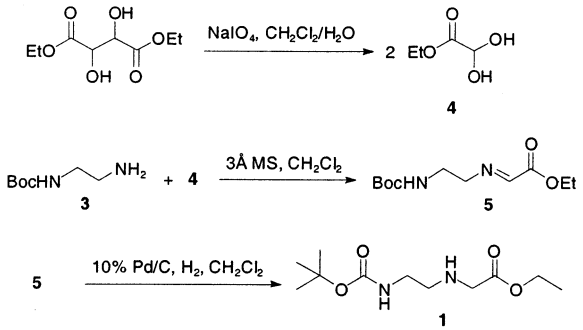
A ubiquitous requirement in the field of peptide nucleic acid (PNA) research[1] is the preparation of monomers for subsequent oligomerization. Although the monomers suitable for both *t*-Boc and Fmoc strategies of solid-phase peptide synthesis are commercially available, they are costly and of limited variety.[2] For researchers developing unusual or modified monomers, there is usually the necessity to prepare *N*-(2-aminoethyl)glycine derivatives. Driven by our own need to prepare PNA monomers, we have developed a reliable, convenient, and scalable route to ethyl *N*-[2-Boc-aminoethyl]glycinate, **1**. We show that compound **1** may be used for PNA monomer synthesis without explicit purification and is a key intermediate in the synthesis of all standard PNA monomers (**2** and Scheme 1), compatible with Merrifield graded acidolysis oligopeptide synthesis.[3]

The "backbone" polymer of PNA is comprised of 2-aminoethylglycine repeat units. This structure is commonly prepared by the reaction of Boc-ethylenediamine[4] (**3**) with a haloacetic acid derivative, most often ethyl bromoacetate.[5] Even when great care is taken, this method invariably produces a mixture of the desired product and varying amounts of the undesired dialkylated amine, often containing unreacted **3** as well. This procedure is therefore inefficient in the use of **3**, and inconvenient to scale-up since chromatography is generally used to purify the crude product.

SCHEME 1



SCHEME 2



Herein, we report a new procedure that efficiently converts **3** to **1** without the need for purification and is therefore easily scalable. The procedure (Scheme 2) is based on the formation of the imine from **3** and ethyl glyoxylate hydrate, itself obtained by oxidative cleavage of diethyl tartrate, followed by reduction of the imine to afford the desired secondary amine **1** without the possibility of overalkylation.

A key factor in the success of this scheme is the method by which ethyl glyoxylate is prepared. A variety of methods for the oxidative cleavage of diethyl tartrate were surveyed. In general, it was found that ethyl glyoxylate was difficult to prepare as the free aldehyde. For instance, NMR analysis of the crude aldehyde prepared by the reaction of periodic acid with diethyl tartrate in reagent grade ether[6] showed almost no sign of the desired aldehyde and contained a considerable amount of the diethyl acetal, presumably due to the presence of ethanol as stabilizer in the ether. The spectrum also showed signals ascribed to unidentifiable oligomeric or polymeric material. When the cleavage was repeated in distilled ether, acetal formation was suppressed, but no increase in the amount of free aldehyde content was observed. Use of dichloromethane as a solvent slowed the reaction and did not improve the quality of the product. The crude aldehyde from any of the above procedures was not competent in imine formation. Given the high reactivity of the free aldehyde,[7] and the observed low reactivity of the polymeric or ethyl acetal form of this aldehyde, it was decided to intentionally prepare the hydrated form. Initially, aqueous sodium periodate supported on silica gel[8] was used; however, this reaction was slower and more cumbersome, especially on

(1) For more about the application, properties, and standard syntheses of PNA, see the following review and references therein: Nielsen, P. E. *Acc. Chem. Res.* **1999**, *32*, 624−630.

(2) *t*Boc- and Fmoc-PNA monomers are available from Applied Biosystems, Foster City, CA 94404.

(3) Dueholm, K. L.; Egholm, M.; Behrens, C.; Christensen, L.; Hansen, H. F.; Vulpius, T.; Petersen, K. H.; Berg, R. H.; Nielsen, P. E.; Buchardt, O. *J. Org. Chem.* **1994**, *59*, 5767−5773.

(4) Kofoed, T.; Hansen, H. F.; Orum, H.; Koch, T. *J. Peptide Sci.* **2001**, *7*, 402−412.

(5) Meltzer, P. C.; Liang, A. Y.; Matsudaira, P. *J. Org. Chem.* **1995**, *60*, 4305−4308.

(6) Kelly, R. K.; Schmidt, T. E.; Haggerty, J. G. *Synthesis* **1972**, 544−545.

(7) Wennerberg, J.; Polla, M.; Frejd, T. *J. Org. Chem.* **1997**, *62*, 8735−8740.

(8) Zhong, Y.-L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622−2624.

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scale-up, since the desired aldehyde hydrate was slightly adsorbed to the silica gel, requiring extensive rinsing with dichloromethane or reduced product recovery. Rinsing with reagent grade ether resulted in acetal formation, while the use of more polar solvents such as ethyl acetate or acetonitrile resulted in the leaching of iodine species into solution. Finally, ethyl glyoxylate hydrate was reliably prepared by the reaction of diethyl tartrate with sodium periodate in 5:1 dichloromethane/water.[9,10]

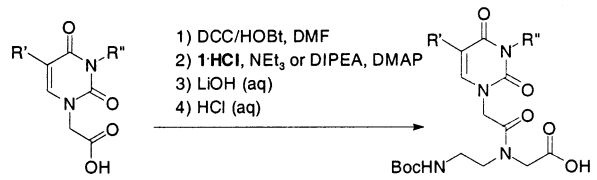
Once pure **4** was in hand, the remainder of the synthesis was easily carried out. Treatment of mono-Boc-ethylenediamine (**3**) with 1.1 equiv of **4** in dichloromethane at 0 °C over 3 Å MS quantitatively afforded the corresponding imine (**5**) within an hour. Filtration of this solution, followed by addition of 0.05 equiv of Pd (10% on activated carbon) and hydrogenation afford the PNA backbone monomer (**1**). The imine solution has also been stored overnight at −20 °C or evaporated and stored at −20 °C with little or no effect on the purity of the final product.

When the hydrogenation was carried out in the presence of molecular sieves in an attempt to avoid the filtration step, the quality of the crude product was substantially decreased, containing a number of unidentified side products.

To demonstrate the scalability of the procedure, **1** has been prepared on 2-, 10-, and 38-g scales. On each scale, the desired product was isolated pure and in essentially quantitative yield. For scale-up, the amounts of reagents and solvents were scaled equally, and reaction times remained the same. The only difference between the scales was the method of hydrogenation. On the 2-g scale, hydrogenation was complete within 4 h of magnetic stirring under a balloon of H_2. However, on the 10-g scale, these conditions afforded only 77% reduction based on NMR analysis of the crude product. By increasing the H_2 pressure to 50 psi and providing vigorous shaking on a Parr hydrogention apparatus, reduction on the 10-g scale was complete within 4 h. On the 38-g scale, too large for our hydrogenation apparatus, reduction was carried out under a stream of H_2, held back by a mercury bubbler. Efficient agitation was achieved by the use of strong magnetic stirring in a Morton-type flask. Under these conditions, reduction was complete within 4 h. In general, the major factor in the rate of the hydrogenation appeared to be the efficiency with which H_2 was transferred to the solution, and so strong agitation is of utmost importance. For this reason, it is recommended (especially on larger scales) that completion of reduction be verified by NMR analysis of a small, filtered, evaporated aliquot of the reaction mixture prior to workup.

When the procedure is carried out as described, the PNA backbone monomer is obtained in highly pure form. However, the crude product can be further purified by dropwise addition of ethereal HCl to an ice-cooled ether solution of crude **1**. The backbone hydrochloride is a stable white solid, which can be recrystallized from acetone, if necessary. As a solid, **1**·HCl is more conveniently stored and dispensed than neutral **1**, which is usually a viscous oil. We have prepared a few PNA monomers from **1**·HCl (Scheme 3), and have observed no disadvantage over the use of the neutral backbone.

SCHEME 3



6a: R' = Me, R" = H
6b: R' = Me, R" = PMB
6c: R' = I, R" = H

2a: 48%
2b: 67%
2c: 61%

In summary, we have developed a new synthesis of the peptide nucleic acid monomer precursor, which is preferable to reported methods due to its efficiency, reduced labor, and especially the elimination of the need for purification by chromatography. Furthermore, we report the preparation, purification, and use of the hydrochloride salt of ethyl *N*-[(2-Boc-amino)ethyl]glycinate, a more convenient form of this important compound.

Experimental Section

Ethyl glyoxylate hydrate,[9] Boc-ethylenediamine,[4] thymin-1-ylacetic acid,[3] N3-PMB-thymin-1-ylacetic acid,[11] and 5-iodouracil-1-ylacetic acid[12] were prepared according to literature methods. Ethereal HCl was prepared by dropwise addition of a large excess of concentrated HCl to an equal volume of concentrated H_2SO_4, and bubbling the gas thus formed through stirred, ice-bath cooled diethyl ether. This reagent was stored at −20 °C and titrated prior to use. Molecular sieves were pulverized and activated at 300 °C under vacuum for 3 days prior to use. All other reagents and solvents were used as supplied, without further purification.

Ethyl *N*-(2-Boc-aminoethyl) Glycinate (1). To an ice-bath cooled solution of ethyl glyoxylate hydrate (**4**, 5.37 g, 44.7 mmol) in CH_2Cl_2 (∼90 mL) was added 3 Å MS (∼5 g), followed by dropwise addition of Boc-ethylenediamine (**3**, 6.50 g, 40.6 mmol) in CH_2Cl_2 (∼10 mL) over ∼10 min. The mixture was stirred at 0 °C for 1 h and then filtered through a short pad of Celite. Evaporation of a few drops of this solution, followed by ^{1}H NMR analysis (600 MHz, $CDCl_3$) revealed complete conversion of **3** to imine **5**: δ 7.60 (s, 1H), 4.93 (br s, 1H), 4.22 (q, J = 7.2 Hz, 2H), 3.64 (t, J = 5.3 Hz, 2H), 3.36 (app q, J = 5.3 Hz, 2H), 1.31 (s, 9H), 1.24 (t, J = 7.2 Hz, 3H). Palladium (10% on activated carbon, 2.16 g, 2.03 mmol) was added to the filtered solution and the mixture was hydrogenated at 50 psi. After 4 h, the mixture was filtered through a hard-packed pad of Celite and rinsed with MeOH under a stream of N_2 (**Caution!** Pd/C is pyrophoric in open air!). The solution was evaporated in vacuo to afford 9.8 g (98%) of the desired product (**1**), a yellow oil that partially crystallized on standing. The spectral data were in agreement with those previously reported.[5]

1·HCl. To an ice-bath cooled solution of **1** (8.71 g, 35.4 mmol) in Et_2O (150 mL) was added ethereal HCl (1.08 M, 35 mL, 37.8 mmol) dropwise over ∼5 min. The mixture was stirred at 0 °C for 1 h, then filtered, rinsed with Et_2O, and dried in vacuo to afford 8.2 g (82%) of **1**·HCl, an air-stable, nonhygroscopic white solid. Mp 121−124 °C dec. ^{1}H NMR (400 MHz, D_2O): δ 4.13 (q, J = 7.3 Hz, 2H), 3.85 (s, 2H), 3.27 (t, J = 5.3 Hz, 2H), 3.07 (t, J = 5.4 Hz, 2H), 1.27 (s, 9H), 1.12 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, D_2O): δ 167.09, 158.30, 81.83, 63.75, 47.70, 36.87,

(9) Bailey, P. D.; Smith, P. D.; Pederson, F.; Clegg, W.; Rosair, G. M.; Teat, S. J. *Tetrahedron Lett.* **2002**, *43*, 1067−1070.

(10) Technical grade ethyl glyoxylate, ∼50% in toluene, is commercially available from Fluka cat. #50705 and Lancaster Synthesis cat. #19207, but this material "exists partly in the polymerized form" (2001/2002 Fluka laboratory chemicals and analytical reagents catalog). We have not yet attempted to synthesize **1** using this product.

(11) Viirre, R. D.; Hudson, R. H. E. *Org. Lett.* **2001**, *3*, 3931−3934.

(12) Hudson, R. H. E.; Li, G.; Tse, J. *Tetrahedron Lett.* **2002**, *43*, 1381−1386.

27.94, 13.58. HRMS exact mass 247.1651, calcd for $C_{11}H_{23}N_2O_4^+$ 247.1652. Anal. Calcd for $C_{11}H_{23}ClN_2O_4$: C, 46.72; H, 8.20; Cl, 12.54; N, 9.91; O, 22.63. Found: C, 46.44; H, 8.45; Cl, 12.70; N, 9.70; O, 22.80.

General Method for the Preparation of PNA Monomers (2) with 1·HCl. To an ice-cooled solution of a nucleobase acetic acid derivative (**6**, 1 equiv) and 1-hydroxybenzotriazole (1.1 equiv) in a minimum amount of dry DMF was added *N*,*N*′-dicyclohexylcarbodiimide (1.1 equiv). The mixture was removed from the ice-bath and stirred for 2 h. The mixture was then cooled, and to it was added a solution containing **1**·HCl (1.1 equiv), TEA or DIPEA (3.3 equiv), and 4-(*N*,*N*-dimethylamino)-pyridine (0.1 equiv) in a minimum amount of DMF (1 g of **1**·HCl is clearly soluble in 6 mL of DMF). The mixture was again removed from the ice-bath and stirred overnight. The mixture was then worked up as previously described[3] to afford the desired PNA monomers in the yields reported in Scheme 3. The spectral data for **2a**[3] and **2c**[12] were in agreement with those previously reported. **2b**: Mp 182 °C dec. ^{1}H NMR (400 MHz, DMSO, 20 °C, 2 rotamers): δ 7.41 (s, ma), 7.36 (s, mi), 7.22 (d, J = 8.7 Hz, 2H), 6.94 (t, J = 5.8 Hz, ma), 6.83 (d, J = 8.7 Hz, 2H), 6.75 (t, J = 5.5 Hz, mi), 4.91 (s, 2H), 4.72 (s, ma), 4.54 (s, mi), 4.17 (s, mi), 3.97 (s, ma), 3.70 (s, 3H), 3.38 (t, J = 6.5 Hz, ma), 3.30 (t, J = 6.9 Hz, mi), 3.18−3.14 (m, ma), 3.03−2.99 (m, mi), 1.80 (s, 3H), 1.36 (s, 9H). ^{13}C NMR (100 MHz, DMSO, 20 °C, 2 rotamers): δ 170.84 (mi), 170.50 (ma), 167.43 (mi), 167.00 (ma), 163.127, 158.48, 155.79 (ma), 155.61 (mi), 151.12, 140.90, 129.41, 129.19, 113.66, 107.47, 78.06 (ma), 77.77 (mi), 55.05, 49.20 (mi), 48.99 (mi), 48.85 (ma), 47.51 (ma), 46.91 (mi), 46.72 (ma), 43.11, 38.04 (ma), 37.58 (mi), 28.22 (mi), 28.15 (ma), 12.59. HRMS exact mass 504.2222, calcd for $C_{24}H_{32}N_4O_8$ 504.2220.

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