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An Improved Synthesis of the Selective Matrix Metalloproteinase Inhibitor, Ro 28-2653

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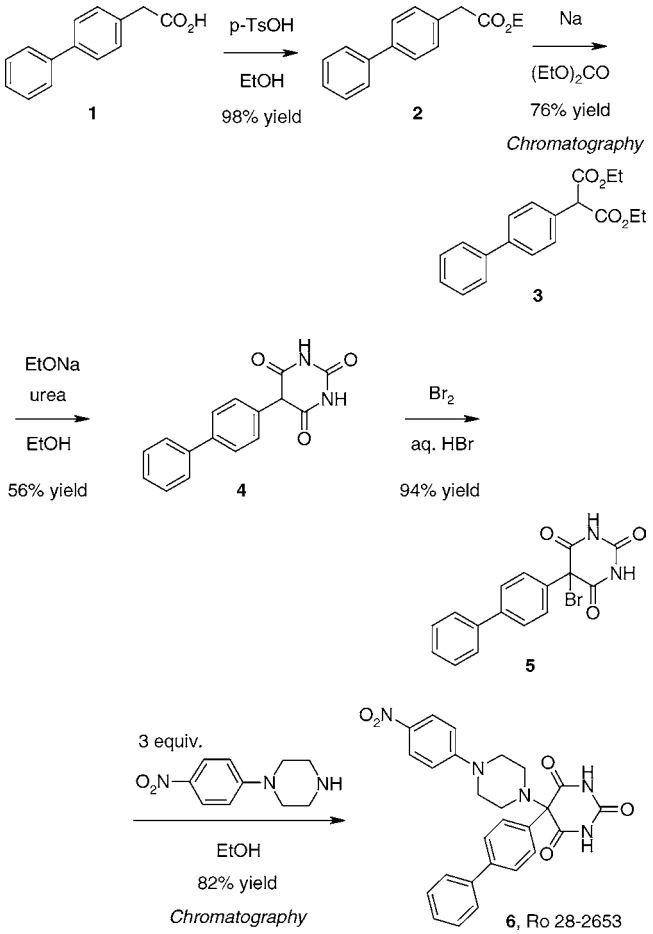
Abstract:

An efficient synthesis of Ro 28-2653, a selective matrix metalloproteinase inhibitor, has been developed. The title compound was prepared in four steps and 76% overall yield from 4-biphenylacetic acid. The key, barbituric acid formation step was significantly improved by using 2-propanol and potassium *tert*-butoxide as the solvent and base, respectively, instead of the typical ethanol and sodium ethoxide combination.

Introduction

Ro 28-2653 (**6**)[1] belongs to a new, barbiturate class of matrix metalloproteinase (MMP) inhibitors with high selectivity for the gelatinases A and B (MMP-2 and MMP-9), which have been implicated in tumor growth and metastasis.[1,2] Preclinically, Ro 28-2653 has been shown to reduce tumor growth and extend survival time of tumor-bearing animals[3] and, more recently, to induce apoptosis in the tumor cells.[4] On the basis of these results and other preclinical data, Ro 28-2653 (**6**) was chosen as a clinical candidate, and consequently, multikilogram quantities of **6** were required for further toxicological and clinical studies. The original medicinal chemistry synthesis was quite reasonable: five steps and 32% overall yield from commercially available biphenylacetic acid (**1**).[5] The major concern prior to initial scale-up by the Kilo Laboratory was the use of column chromatography to isolate malonate **3** and the final product, **6**. They were able to eliminate the chromatographic purification on a small scale, but byproduct formation significantly increased upon scale-up to 100 g quantities, resulting in diminished overall yield (less than 10%) and unacceptable purity of the final product. This triggered our investigation of the process. Herein, we report the improved, four-step synthesis of **6**, which gave the title compound in 76% overall yield and >99.5% purity.



Results and Discussion

Malonate Formation Step. In the original procedure for the conversion of **2** to malonate **3**,[5] 1.1 equiv of sodium was added portionwise to a solution of **2** in 8.5 vol of diethyl carbonate (i.e., 8.5 mL per g of **2**), and the mixture was heated to 120 °C for 3 h. Then, the relatively low-melting malonate **3** (mp 51−53 °C) was isolated by chromatography. The Kilo Lab subsequently replaced the metallic sodium with sodium ethoxide because of the obvious safety concern and eliminated the chromatography. Instead, the crude product, obtained after extractive workup, was heated to 220 °C under high vacuum to remove the residual diethyl carbonate, and the resulting oily residue was directly used in the following barbituric acid formation step without further purification. The use of the crude material may have contributed, at least partly, to the increased byproduct formation. Since removal of the excess diethyl carbonate is awkward because of its

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(1) Grams, F.; Brandstetter, H.; D'Alò, S.; Geppert, D.; Krell, H.-W.; Leinert, H.; Livi, V.; Menta, E.; Oliva, A.; Zimmermann, G. *Biol. Chem.* **2001**, *382*, 1277.
(2) Foley, L. H.; Palermo, R.; Dunten, P.; Wang, P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 969.
(3) Lein, M.; Jung, K.; Ortel, B.; Stephan, C.; Rothaug, W.; Juchem, R.; Johannsen, M.; Deger, S.; Schnorr, D.; Loening, S.; Krell, H.-W. *Oncogene* **2002**, *21*, 2089.
(4) Mangoldt, D.; Sinn, B.; Lein, M.; Krell, H. W.; Schnorr, D.; Loening, S. A.; Jung, K. *Apoptosis* **2002**, *7*, 217.
(5) Bosies, E.; Esswein, A.; Grams, F.; Krell, H.-W.; Menta, E. W.O. Patent 9723465, 1997.

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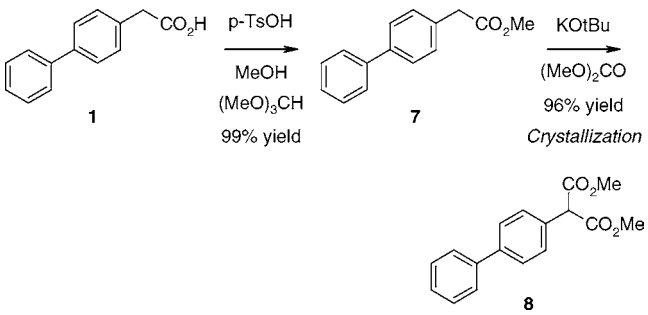
Table 1. **Preparation of barbituric acid 4**

	reaction conditions			HPLC analysis and yield				
				crude **4**[c]		purified **4**[d]		
entry	temp[a] (°C)	*t*-BuOK (equiv)	addition time (h)[b]	purity (%)[e]	**9** (%)[e]	yield (%)	purity (%)[e]	**9** (%)[e]
1	70	2.0	1.0	76.7	3.8	73.0	96.6	0.7
2	80 ± 2	2.0	1.0	89.6	6.5	91.0	94.4	2.2
3	80 ± 2	2.0	2.7	91.9	2.4	88.1	99.5	0.3
4	80 ± 2	2.0	4.0	90.5	2.2	90.3	99.7	0.2
5	80 ± 2	2.15	4.3	90.8	6.4	90.0	97.5	2.4
6	80 ± 2	1.5	4.0	90.9	1.3	90.4	99.6	0.2
7	80 ± 2	1.2	4.0	89.2	1.1	88.8	99.8	0.1
8	80 ± 2	1.15	3.8	90.0	1.3	89.1	99.5	0.2
9	80 ± 2	1.0	4.0	86.7	0.8	84.1	99.6	0.2

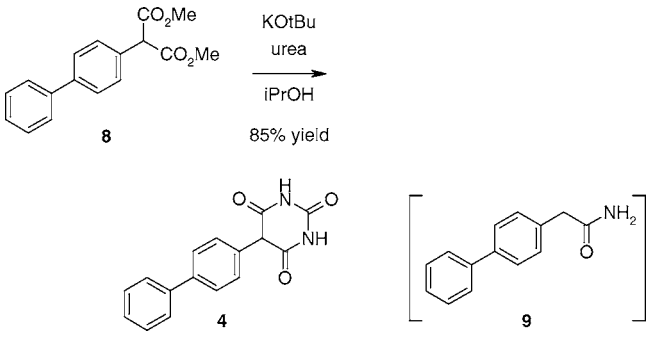
[a] Temperature of the reaction mixture. For the 80 °C reaction, it was necessary to remove some of the THF from the reaction mixture by distillation to maintain the desired reaction temperature. [b] A syringe pump was used for the addition of a *t*-BuOK solution in THF over the given time frame. The mixture was heated to 70 °C for a total of 8 h (entry 1) or 80 °C for a total of 6 h (entries 2−9) to achieve essentially complete reaction. [c] Crude **4** was obtained by the addition of dilute hydrochloric acid to the reaction mixture and collecting the resulting precipitate by filtration. [d] Purified **4** was obtained by heating a suspension of the crude product in 9:1 ethyl acetate:DMF to reflux for 30 min, then collecting the solid by filtration at 46 °C. [e] Area % at 265 nm.

relatively high boiling point (126−128 °C), the reaction of methyl ester **7** with dimethyl carbonate (bp 90 °C) was investigated. Thus, methyl ester **7**, prepared in a standard manner as was the ethyl ester, was dissolved in 3 vol of dimethyl carbonate and potassium *tert*-butoxide (2.2 equiv; as a 1.66 M THF solution) was added. The amount of base was increased from the original 1.1 equiv to facilitate the reaction, which was complete within an hour at 60 °C. After extractive workup, crystalline dimethyl malonate **8** was obtained by trituration with hexane in 95% overall yield from **1**. As this compound was found to be readily prepared in high yield and has a more desirable melting point (98−99 °C) than the diethyl malonate **3**, dimethyl malonate **8** was considered as a starting material, and our focus shifted to the more troublesome step, formation of the barbituric acid **4**.



Barbituric Acid Formation Step. The Kilo Lab obtained barbituric acid **4** from **3** in 36% yield on a 100-g scale by applying the standard condensation protocol with urea (1.5 equiv) and sodium ethoxide (2 equiv) in ethanol at reflux. Heavy precipitation of the sodium salt of the product necessitated a large volume of ethanol (27 vol). The major byproduct obtained from this reaction was 4-biphenylacetamide (**9**), which was difficult to remove from the product. To minimize the formation of this byproduct, several other solvents and bases were examined. A system using potassium *tert*-butoxide in THF as the base and 2-propanol as the solvent was found to be the best in terms of minimizing the impurity formation and reaction time, which in turn provided the highest isolated yield of the product **4** from **3**. Moreover, the potassium salt of the product did not precipitate as heavily as the sodium salt, thus enabling a reduction in the amount of solvent required (from the original 27 vol of ethanol, to 6 vol of 2-propanol). Under these conditions, the amount of urea can be reduced to 1.25 equiv from the original 1.5 equiv without compromising the yield and purity of the product. This new procedure worked well with both diethyl malonate **3** and dimethyl malonate **8**.

To maximize the product yield and minimize the formation of byproduct **9**, several reaction parameters (i.e., temperature, duration of base addition, and amount of base) were examined (Table 1) using the preferred starting material, dimethyl malonate **8**.



(a) Temperature (entries 1 and 2). An 18% decrease in yield was observed by decreasing the reaction temperature from ca. 80 to 70 °C. The 70 °C reaction resulted in increased amounts of byproducts, as determined by HPLC and TLC analysis, when compared to running the reaction at 80 °C.

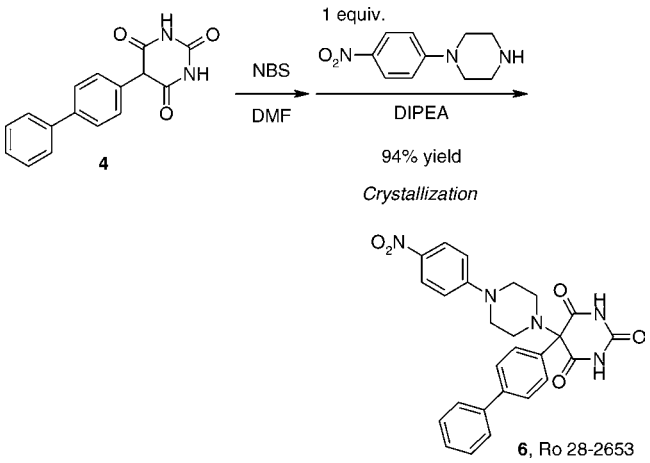
(b) Duration of Base Addition (entries 2−4). The slower the addition of base, the lower the amount of byproduct **9** that formed (6.5% with 1 h addition and 2.2% with 4 h addition).

(c) Amount of Base (entries 4−9). By increasing the amount of base by 7.5%, from 2 equiv to 2.15 equiv (entries 4 and 5), the amount of **9** increased ca. 3-fold, from 2.2% to 6.4%. Virtually identical yields and qualities of **4** were obtained using 1.15−2.0 equiv of *t*-BuOK (entries 4, 6−8). When just one equivalent of the base was used, the isolated yield dropped by ca. 5%, presumably, due to incomplete reaction.

On the basis of these findings, a trial run was performed on a 100-g scale. Thus, a mixture of malonate **8**, urea (1.25 equiv) and 2-propanol (6 vol) were heated to reflux (ca. 85 °C) and a 1.66 M solution of *t*-BuOK in THF (1.2 equiv) was added over 4.5 h. Since the THF was not removed by distillation in this experiment, the temperature of the reaction mixture, after the complete addition of the base, was ca. 77 °C. During the addition, precipitates formed in the *t*-BuOK solution in the addition funnel, which made the continuous addition of the base difficult. Thus, the base solution was added to the reaction mixture in small portions over the stated time period. Crude **4** obtained by filtration (containing 0.9%

of byproduct **9**) was not dried but was suspended in ethyl acetate, and the mixture was heated to reflux to remove water azeotropically. After cooling the suspension to 30 °C, the solid was collected by filtration to give **4** in 85% yield and 99.5% purity (containing 0.1% of **9**).

Preparation of Ro 28-2653 from Barbituric Acid. Because the use of bromine in the original procedure[5] was undesirable for scale-up, bromination with *N*-bromosuccinimide (NBS) was investigated. The reaction of barbituric acid **4** in DMF with one equivalent of NBS was spontaneous, gave a very clean product, and was mildly exothermic. Thus, on scale-up, a DMF solution of NBS was added slowly to control the temperature. Addition of one equivalent each of 1-(4-nitrophenyl)piperazine and *N*,*N*-diisopropylethylamine (DIPEA) to the thus obtained solution of bromide **5** cleanly produced the product **6**, thus eliminating the isolation of the relatively unstable bromide **5**. Reducing the amount of the piperazine from the original 3 equiv to 1 equiv was not only more economical but also led to a simpler procedure for the isolation of the product; the product was directly precipitated from the reaction mixture by the addition of water. The resulting thick suspension was subsequently heated to 60 °C and then cooled to room temperature prior to filtration. This brief heating of the suspension resulted in a much faster filtration. The crude **6** thus obtained was recrystallized from acetic acid to give **6** in 94% yield from **4** and 99.7% purity.



Conclusions

An efficient, four-step synthesis of Ro 28-2653, a selective matrix metalloproteinase inhibitor, has been developed. The diethyl malonate **3** used in the original process was replaced with dimethyl malonate **8**, which was easier to prepare and isolate in pure form by recrystallization. An improved protocol was also established for the formation of barbituric acid, in which a small excess of potassium *tert*-butoxide was slowly added in portions to a mixture of dimethyl malonate **8** and urea in 2-propanol at reflux. This procedure minimized the formation of decarbonylated byproducts and gave barbituric acid **4** in high yield and purity. Although this study focused exclusively on **4**, this new improved protocol should be generally useful for the preparation of other barbituric acid derivatives. Bromination of **4** was achieved with one equivalent of NBS in DMF, instead of the original procedure that utilized bromine in aqueous HBr. The resulting mixture was directly treated with one equivalent of both 1-(4-nitrophenyl)piperazine and DIPEA to give the title compound **7**. As a consequence, the isolation of the relatively unstable bromide **5** and the use of large excess of the piperazine (i.e., 3 equiv) were avoided. Thus, the title compound, Ro 28-2653 (**6**), was prepared in four steps and 76% overall yield from 4-biphenylacetic acid (**1**).

Experimental Section

General. All reagents and solvents were obtained from commercial suppliers and used without further purification. The HPLC analysis data is reported in area %, not adjusted to weight %.

HPLC conditions: Column: Zorbax Rx-C18, 10 μm, 5 × 250 mm; mobile phase: 20−100% acetonitrile (+ 0.1% TFA) over 20 min at 1.5 mL/min; detection: UV, 265 nm.

Methyl (4-Biphenyl)acetate (7). A mixture of 4-biphenylacetic acid (**1**, 100 g, 471 mmol), *p*-TsOH monohydrate (10 g, 52.6 mmol) and methanol (650 mL) was heated to reflux for 1 h, and trimethyl orthoformate (50 mL, 457 mmol) was added. After an additional 2 h of reflux, TLC analysis indicated complete reaction. The mixture was concentrated to dryness under reduced pressure. The resulting residue was dissolved in a 1:1 mixture of hexane and *tert*-butyl methyl ether (400 mL), and the resulting solution was washed with saturated $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated to dryness under reduced pressure to give 106 g (99.3% yield) of **7** as a colorless oil (lit.[6] mp 19−21 °C). This material was used directly in the next step.

Dimethyl (4-Biphenyl)malonate (8). To a mixture of **7** (35.0 g, 155 mmol) and dimethyl carbonate (105 mL, 1.25 mol) was added a 1.66 M solution of *t*-BuOK in THF (195 mL, 324 mmol) over 10 min, and the mixture was heated to 60 °C for 1 h. TLC analysis indicated complete reaction. The reaction was quenched by the addition of acetic acid (27 mL, 472 mmol). The resulting mixture was diluted with ethyl acetate (80 mL) and washed with water (200 mL) and then with a mixture of water (100 mL) and saturated NaCl solution (50 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness under reduced pressure and then under vacuum to completely remove the dimethyl carbonate. The resulting solid was triturated with hexane (100 mL), collected by filtration, and dried by suction to give 42.2 g (95.9%) of **8**[2] as a white crystalline solid: mp 98−99 °C. ^{1}H NMR ($CDCl_3$): δ 7.59 (m, 4 H); 7.47 (m, 4 H); 7.35 (m, 1 H); 4.70 (s, 1 H); 3.79 (s, 6H).

5-(4′-Biphenyl)barbituric Acid (4). A mixture of **8** (100 g, 352 mmol), urea (26.4 g, 440 mmol), and 2-propanol (600 mL) was heated to reflux (ca. 85 °C), and a 1.66 M solution of *t*-BuOK in THF (254 mL, 422 mmol) was added over 4.5 h in small portions, while maintaining the mixture at reflux. After completion of the addition, the mixture was heated to reflux (ca. 77 °C) for an additional 2 h. After heating was discontinued, a mixture of concentrated HCl (58.2 mL, 703 mmol) and water (200 mL) was slowly added to the warm mixture, and the organic solvents (THF and

(6) Wright, S. W.; Hageman, D. L.; McClure, L. D. *J. Org. Chem.* **1994**, *59*, 6095.

2-propanol) were removed under reduced pressure. The resulting aqueous mixture was diluted with water (500 mL). The resulting precipitate was collected by filtration and washed with water (500 mL). The wet solid thus obtained was then suspended in ethyl acetate (1 L), and the mixture was heated to reflux for 30 min with continuous removal of water using a Dean−Stark trap. After cooling to 30 °C, the solid was collected by filtration, washed with ethyl acetate (300 mL), and dried by suction to give 84.0 g (85.2% yield) of **4** as a white solid;[5] 99.5% pure by HPLC analysis. ^{1}H NMR (DMSO-d_6): δ 11.62 (bs, ca. 1.7 H); 10.75 (bs, ca. 0.6 H); 7.63 (m, 4H); 7.45 (m, 2 H); 7.38 (m, 3 H); 4.92 (bs, ca. 0.7 H). Compound **4** exists as a tautomeric mixture in DMSO solution.[7]

5-(4′-Biphenyl)-5-[*N*-(4-nitrophenyl)piperazinyl]barbituric Acid (6). A suspension of **4** (84.0 g, 300 mmol) in DMF (550 mL) was stirred at room temperature for 30 min to dissolve most of the solids. After cooling to 10 °C, a solution of NBS (55.0 g, 300 mmol) in DMF (150 mL) was added over 10 min, while maintaining the temperature of the reaction mixture between 5 and 10 °C. The addition funnel was rinsed with DMF (20 mL), and the rinse was added to the reaction mixture. After stirring at 3−7 °C for 16 min, 1-(4-nitrophenyl)piperazine (62.1 g, 300 mmol) was added in one portion. The funnel and the inside-walls of the reaction flask were rinsed with DMF (25 mL), and the rinse was added to the reaction mixture. Then DIPEA (52.2 mL, 300 mmol) was added. The cooling bath was removed, and the mixture was stirred at room temperature for 1 h. TLC analysis indicated complete reaction. The mixture was poured into water (2.88 L), and the resulting suspension was briefly heated to 60 °C. After cooling to room temperature, the yellow solid was collected by filtration, washed with water (1 L), and dried by suction in the absence of light. The wet, crude product (375 g) thus obtained was suspended in acetic acid (1 L), and the mixture was heated to 108 °C for 15 min. After cooling to 23 °C, the solid was collected by filtration, washed with acetic acid (200 mL), and dried by suction overnight. The resulting solid was further dried at 58 °C/150 mmHg to give 145 g (93.8% yield) of **6** as a yellow solid;[1,5] 99.7% pure by HPLC analysis.

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(7) Jovanovic, M. V.; Biehl, E. R. *Heterocycles* **1986**, *24*, 3129.