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An efficient total synthesis of a sphingosine-1-phosphate receptor agonist KRP-203

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Abstract

An efficient total synthesis of the $S1P_1$ agonist, KRP-203, is described. The key step involves the conjugate addition of diethyl acetamidomalonate to a styrene compound to give the core structure of KRP-203. Multigram quantities of the targeted KRP-203, sufficient for several biological studies, were obtained in six steps with an overall yield of 58%.

1. Introduction

In recent years, many immunosuppressants have been developed, some of which have been introduced in clinical organ transplantation. FTY720 (**1**, Fig. 1),[1–6] a novel immunomodulator series, is the first sphingosine-1-phosphate (S1P) receptor agonist[7,8] that has proven effective in animal models of transplantation and autoimmunity[9,10] and has achieved promising results in phase 1 and phase 2 trials of renal transplantation in humans.[11] FTY720 (**1**) exhibits immunosuppressive activity by inhibiting the response of naïve and mature lymphocytes to S1P, and inducing the sequestration of circulating naïve and mature lymphocytes from the blood and peripheral tissues into secondary lymphoid organs.[12,13] Its effect relates to the modulation of lymphocyte trafficking. A novel immunomodulator KRP-203 (**2**, Fig. 1),[14–16] which is an analog of **1**, is a S1P receptor agonist with increased selectivity on the $S1P_1$ receptor over the $S1P_3$ receptor. The $S1P_3$ receptor may be associated with clinically observed adverse events of **1** including a transient, asymptomatic bradycardia.[17,18] As part of research into structure–activity relationships (SAR) focused on attenuating the adverse events, multigram quantities of **2** were required along with an efficient synthesis process for construction of its analogs. Recently we reported an easily accessible synthesis of **2** by a Pd-catalyzed cross-coupling reaction;[19] however, the synthetic methodology is not suitable for the preparation of its related analogs having a variety of hydrophilic parts. An extensive SAR study on the hydrophilic part of **1** has been conducted to lead to a discovery of some unique compounds.[5] In our continuing synthetic studies on **2** and its analogs having a modified hydrophilic part, we developed a new procedure which is characterized by an addition reaction between carbonyl compounds having activated methylenes and styrenes using cesium carbonate in dimethylsulfoxide (DMSO). The method enables us not only to easily synthesize various hydrophilic part modified analogs of **2** but also to allow for an easy preparation of multigram quantities of **2**.

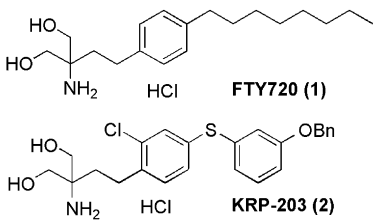

Figure 1. Structures of FTY720 (**1**) and KRP-203 (**2**).

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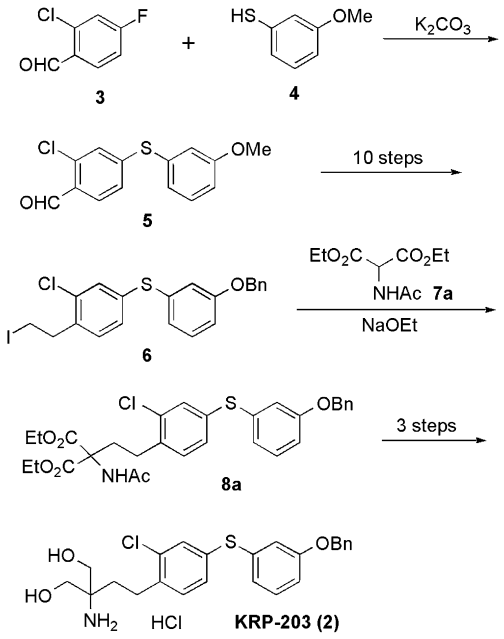
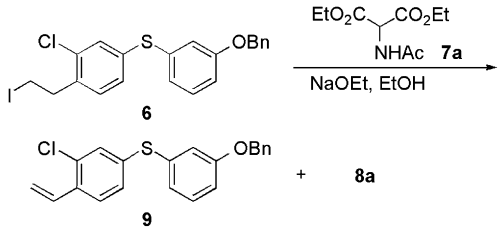
2. Results and discussion

2.1. Development of conjugate addition

While our first attempted route based on the reported route for **2**[14,16] was capable of supplying small amounts of the desired product, it suffered from the following disadvantages: (i) multistep synthesis with low overall yields; (ii) low yield of diethyl acetamidomalonate (**7a**, DEAM) coupling reaction (Scheme 1; **6**→**8a**, <40%). In the synthesis of **1**,[2–5] the DEAM coupling reaction with 1-(2-iodoethyl)-4-octylbenzene proceeded smoothly to give the coupling compound. In the synthesis of **2**, however, the coupling reaction gave the styrene **9** predominantly and the coupling product **8a** was produced in only low yield (Scheme 2).



Scheme 1. Our first attempted route based on the reported route for KRP-203.



Scheme 2. Issue of DEAM coupling to iodoethylbenzene (**6**).

Based on the above-mentioned results, we hypothesized that the styrene **9** may act as an intermediate in the DEAM coupling reaction with **6**. After screening several bases and solvents in the DEAM coupling reaction with **9**, we found cesium carbonate in DMSO to be effective for this condensation process, as summarized in Table 1. Other solvents such as DMF and THF were ineffective (entries 2 and 3) and, in the absence of base, no product was observed (entry 4). Other bases such as potassium *tert*-butoxide[20] and sodium *tert*-butoxide[21] also gave compound **8a** in moderate yields (entries 5 and 6).

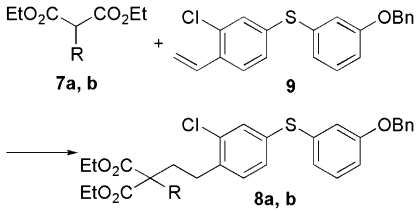
We next set out to define the scope of the coupling reaction with **9** by using methyl malonate **7b** as a simple carbonyl compound to optimize the reaction conditions (entries 10 and 11). Addition of 2.4 equiv of methyl malonate **7b** to 1 equiv of styrene **9** and 1.2 equiv of cesium carbonate in DMSO suspension gave a 96% yield of the coupling product **8b** (entry 10). In contrast, a reaction employing 1.2 equiv of styrene **9**, 1.2 equiv of cesium carbonate, and 1 equiv of malonate afforded **8b** in only 45% yield, along with 52% recovery of **9** (entry 11). These results suggest that the malonate may act as a hydride donor. It is noteworthy that no regioisomer was obtained in any of the styrene coupling reactions. The yield of **8a** and **8b** was significantly high when DMSO was degassed by purging with nitrogen for 15 min prior to use. When cesium carbonate was used as catalyst (0.1 equiv), only a trace amount of the desired product (<5%) was detected. No reactions were observed when 5 equiv of galvinoxyl as radical scavenger was added to the reaction mixture (entries 9 and 12). And the side-product **11**[22] (<30%) was obtained when DEAM (**7a**) was used (Scheme 3). The precise reaction mechanism remains unclear at present, but the above-mentioned findings indicate that the addition of compound **7a** to styrene **9** involves some kinds of free radical intermediates (Scheme 3).[23]

2.2. Synthesis of KRP-203

With the desired styrene reaction in hand, we turned our attention to the synthesis of **2** (Scheme 4). The reaction between 2-chloro-4-fluorobenzaldehyde (**3**) and 3-mercaptophenol (**12**) was selected because of their commercial availability. We were gratified to see that the desired addition progressed smoothly. However, this coupling reaction turned out to be exothermic and therefore difficult to control on a large scale (Table 2, entry 2). To resolve this issue, we developed a new procedure. 3-Mercaptophenol (**12**) was charged slowly into the preheated reaction mixture at 50 °C. This process allows good control of the exothermic behavior. The optimized procedure can be used on a 30 mmol scale to consistently give compound **13** in 98% isolated yield (entry 3).

Subsequent treatment with benzyl bromide in the presence of potassium carbonate afforded the desired aldehyde **14** with 98% yield. On the basis of this study, we performed a one-pot synthesis of compound **14** directly from **3** in 67% yield. This product **14** was then converted to styrene **9** under Wittig or Peterson olefination conditions. The Peterson olefination route was more efficient because the intermediate alcohol can be used without any purification. Subsequent addition of potassium *tert*-butoxide afforded the styrene **9** in 88% yield. To complete the synthesis of KRP-203 (**2**), compound **8a** was reduced with calcium borohydride to afford acetamidopropanediol **15**. Subsequently, deprotection with hydrochloric acid provided **2** in six linear steps from commercially available starting materials.

Table 1
Reaction condition screening[a]

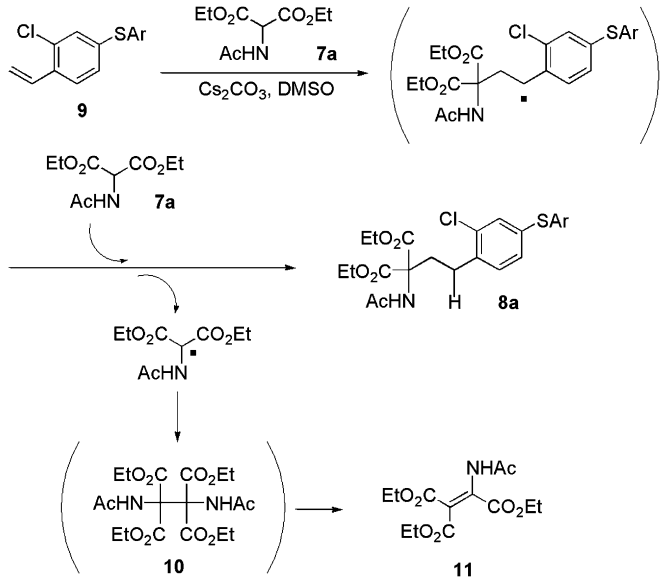


Entry	R	Base	Solvent[b]	Product	Yield[c] (%)
1	**7a**, NHAc	Cs_2CO_3	DMSO	**8a**	83
2	**7a**, NHAc	Cs_2CO_3	DMF	**8a**	5
3	**7a**, NHAc	Cs_2CO_3	THF	**8a**	NR[f]
4	**7a**, NHAc	No base	DMSO	**8a**	NR[f]
5	**7a**, NHAc	KO^{t}Bu	DMSO	**8a**	49
6	**7a**, NHAc	NaO^{t}Bu	DMSO	**8a**	36
7	**7a**, NHAc	KO^{t}Bu	THF/EtOH (1:1)	**8a**	NR[f]
8	**7a**, NHAc	CsOAc	DMSO	**8a**	NR[f]
9[d]	**7a**, NHAc	Cs_2CO_3	DMSO	**8a**	NR[f]
10	**7b**, Me	Cs_2CO_3	DMSO	**8b**	96
11[e]	**7b**, Me	Cs_2CO_3	DMSO	**8b**	45[g]
12[d]	**7b**, Me	Cs_2CO_3	DMSO	**8b**	NR[f]

[a] Reaction conditions: to a solution of 2.4 equiv of malonate **7a** or **7b** and 1.2 equiv of base in DMSO was added a solution of 1 equiv of styrene **9** in DMSO at room temperature. The reaction mixture was stirred at 35 °C[24] overnight under a nitrogen atmosphere in an oil bath (bath temperature).
[b] All solvents were degassed by purging with nitrogen for 15 min prior to use.
[c] Isolated yields after silica gel column chromatography.
[d] Galvinoxyl of 5 equiv as radical scavenger was added to the reaction mixture.
[e] Malonate/Cs_2CO_3/styrene molar ratio=1:1.2:1.2.
[f] NR=no reaction, starting material remains unchanged.
[g] Yield based on malonate **7b**; styrene **9** (52%) recovered.

2.3. Scope of the addition of malonates to styrene

Our unique coupling reaction was broadly applicable to a variety of carbonyl compounds with styrene **9** to prepare analogs of **2**. Six examples of styrene **9** coupling reaction with carbonyl compounds are summarized in Table 3. We were pleased to find that these substrates were generally effective and several functional groups were well tolerated. For example, 2-isopropylmalonate **7c** (entry 1), 2-methoxymalonate **7d** (entry 2), and 2-fluoromalonate **7e** (entry 3) gave the corresponding adducts in good to moderate yields. And 2,2-dimethyl-5-nitrodioxane **7f**,[25–27] which has an acidic proton (pK_a 18 in DMSO),[28–30] also reacted with styrene **9** (entry 4). Finally, amino acid derivatives (**7g** and **7h**) were well tolerated (entries 5 and 6).



Scheme 3. Plausible reaction mechanism.

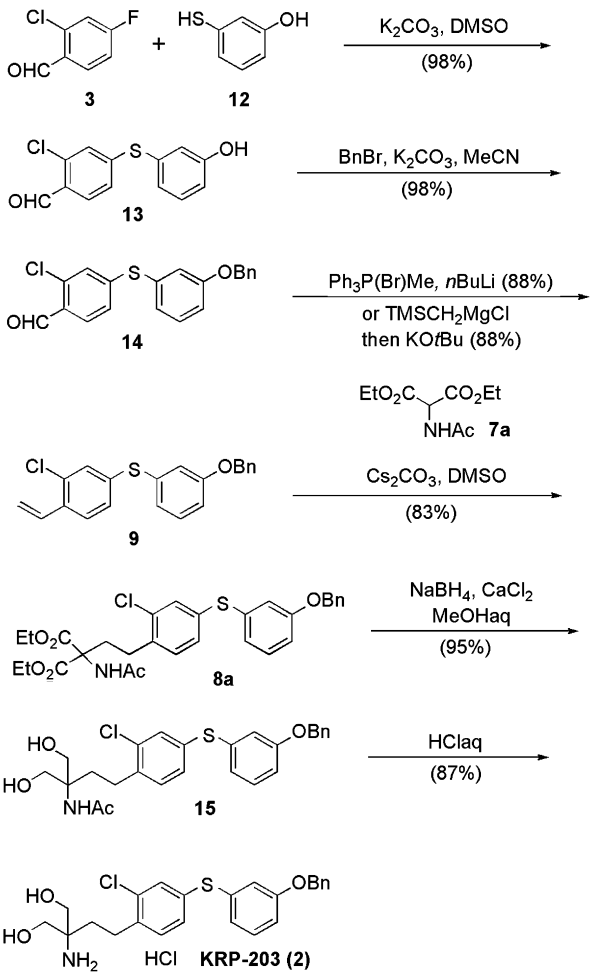
3. Summary

A highly efficient procedure for the synthesis of KRP-203 on a multigram scale was developed. Scheme 4 summarizes the new route for KRP-203 synthesis from a commercially available compound. This process features conjugate addition of malonates, amino acid derivatives, or nitro compounds to styrenes in DMSO in the presence of cesium carbonate.

4. Experimental

4.1. General

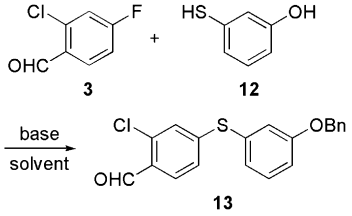
All reactions were carried out in oven-dried glassware under a positive pressure of nitrogen. Anhydrous acetonitrile, dimethylsulfoxide, and tetrahydrofuran were purchased from Aldrich and used without further purification. Flash column chromatography was performed using Biotage SP1™ system. ^{1}H NMR spectra were recorded on a Bruker Avance 400



Scheme 4. Concise synthesis of KPR-203 (**2**).

(400 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to TMS as an internal standard. Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). IR spectra were recorded on a PerkinElmer FT-IR Spectrum One spectrometer. Melting points were obtained on a Büchi 535 melting point apparatus and are uncorrected.

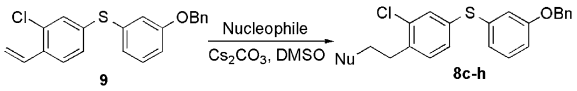
Table 2
Reaction condition screening for the synthesis of **13**[a]



Entry	Base	Solvent	Temp (°C)	Yield (%)	Reaction scale
1	NaH	DMSO	45	73	6 mmol in 60 mL
2	NaH	DMSO	45	33	110 mmol in 260 mL
3	K_2CO_3	DMSO/THF	50	98	32 mmol in 110 mL

[a] Yields given for products isolated after silica gel column chromatography.

Table 3
Scope of the addition of malonates to styrene **9**[a]



Entry	Nucleophile	Product	Yield (%)
1	**7c**	**8c**	43
2	**7d**	**8d**	79
3	**7e**	**8e**	55
4	**7f**	**8f**	37
5	**7g**	**8g**	58
6	**7h**	**8h**	68

[a] Reaction conditions: to a solution of 2.4 equiv of malonate **7a** or **7b** and 1.2 equiv of cesium carbonate in DMSO was added a solution of 1 equiv of styrene **9** in DMSO at room temperature. The reaction mixture was stirred at 35 °C[24] overnight under an N_2 atmosphere in an oil bath (bath temperature). DMSO was degassed by purging with nitrogen for 15 min prior to use. Yields (average of two runs) were given for products isolated after silica gel column chromatography.

Elemental analyses were performed on a Yanako CHN coder MT-5 analyzer. High resolution mass spectra (HRMS) were obtained on a Waters LCT Premier XE-ESI mass spectrum. Most of the reactions were monitored by thin layer chromatography on 0.25 mm E. Merck silica gel plates (60 F254), visualized with UV light, and stained with 5% ethanolic phosphomolybdic acid or *p*-anisaldehyde solution.

The following compounds in this paper are known compounds: 2-amino-2-(2-{4-[3-(benzyloxy)phenylthio]-2-chlorophenyl}ethyl)propane-1,3-diol hydrochloride (KRP-203, **2**),[14,16] 2,2-dimethyl-5-nitro[1,3]dioxane (**7f**),[25–27] 2-acetylamido-3-ethoxycarbonyl-but-2-enedioic acid diethyl ester (**11**).[22]

4.2. General procedure for malonate coupling with styrene

A 50-mL, two-necked, round-bottomed flask equipped with a Teflon-coated magnetic stir bar, a rubber septum, and an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with ethyl acetamidomalonate **7a** (444 mg, 2.05 mmol, 2.4 equiv) and 6 mL of anhydrous DMSO. To the

solution was added cesium carbonate (333 mg, 1.02 mmol, 1.2 equiv) at room temperature and the resulting mixture was stirred at room temperature for 30 min. To the reaction mixture was slowly added styrene **9** (300 mg, 0.85 mmol, 1 equiv, in 2.5 mL of anhydrous DMSO) at room temperature, and was then stirred at 35 °C in an oil bath (bath temperature) overnight. The reaction mixture was cooled to 0 °C in an ice-water bath, and 10 wt % aqueous citric acid was added to the reaction mixture. The aqueous suspension was extracted with dichloromethane (3×20 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from EtOAc/hexane, 13:87 to EtOAc only) to give 403 mg (83%) of **8a** as a colorless oil.

*4.2.1. 2-Acetamido-2-(2-{4-[3-(benzyloxy)phenylthio]-2-chlorophenyl}ethyl)malonic acid diethyl ester (**8a**)*

^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.26 (t, 6H, J=7.1 Hz), 2.05 (s, 3H), 2.53–2.58 (m, 2H), 2.61–2.67 (m, 2H), 4.19–4.28 (m, 4H), 5.02 (s, 2H), 6.84 (s, 1H), 6.86–6.94 (m, 3H), 7.09–7.15 (m, 2H), 7.23 (t, 1H, J=8.0 Hz), 7.29–7.41 (m, 6H); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 14.0, 23.0, 27.5, 32.0, 62.7, 66.2, 70.1, 114.2, 117.3, 123.6, 127.5, 128.1, 128.6, 129.6, 130.2, 130.9, 131.5, 134.5, 135.1, 136.2, 136.5, 137.2, 159.3, 167.9, 169.1; IR (ATR): 1736, 1678, 1474, 1192, 1016 cm^{-1}. Anal. Calcd for $C_{30}H_{32}ClNO_6S\cdot 1/3H_2O$: C, 62.54; H, 5.72; N, 2.43. Found: C, 62.46; H, 5.63; N, 2.73.

*4.2.2. 2-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-2-methylmalonic acid diethyl ester (**8b**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.26 (t, 6H, J=7.1 Hz), 1.52 (s, 3H), 2.09–2.14 (m, 2H), 2.65–2.69 (m, 2H), 4.20 (q, 4H, J=7.1 Hz), 5.02 (s, 2H), 6.86–6.94 (m, 3H), 7.15 (s, 2H), 7.23 (t, 1H, J=8.0 Hz), 7.31–7.40 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.1, 19.9, 28.4, 35.6, 53.5, 61.3, 70.1, 114.1, 117.3, 123.6, 127.5, 128.1, 128.6, 129.7, 130.1, 130.9, 131.6, 134.4, 134.8, 136.3, 136.6, 138.2, 159.3, 172.0; IR (ATR): 1727, 1586, 1474, 1225 cm^{-1}. Anal. Calcd for $C_{29}H_{31}ClO_5S\cdot 1/3H_2O$: C, 65.34; H, 5.99. Found: C, 65.44; H, 5.85.

*4.2.3. 2-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-2-isopropylmalonic acid diethyl ester (**8c**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.03 (d, 6H, J=6.9 Hz), 1.30 (t, 6H, J=7.1 Hz), 2.11–2.15 (m, 2H), 2.41 (q, 1H, J=7.1 Hz), 2.64–2.68 (m, 2H), 4.24 (q, 4H, J=6.9 Hz), 5.01 (s, 2H), 6.86–6.94 (m, 3H), 7.14 (s, 2H), 7.22 (t, 1H, J=7.9 Hz), 7.31–7.38 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2, 18.6, 28.8, 32.2, 33.9, 60.9, 61.6, 70.1, 114.1, 117.2, 123.5, 127.5, 128.1, 128.6, 129.7, 130.1, 131.0, 131.6, 134.4, 134.7, 136.4, 136.5, 138.6, 159.3, 170.9; IR (ATR): 1721, 1586, 1474, 1242, 1221, 1020 cm^{-1}. Anal. Calcd for $C_{31}H_{35}ClO_5S$: C, 67.07; H, 6.35. Found: C, 66.91; H, 6.18.

*4.2.4. 2-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-2-methoxlmalonic acid dimethyl ester (**8d**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.34–2.38 (m, 2H), 2.67–2.71 (m, 2H), 3.44 (s, 3H), 3.81 (s, 6H), 5.02 (s, 2H), 6.87–6.95 (m, 3H), 7.14 (s, 2H), 7.23 (t, 1H, J=7.9 Hz), 7.31–7.39 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.1, 32.5, 52.9, 53.6, 70.1, 84.6, 114.1, 117.4, 123.7, 127.5, 128.1, 128.6, 129.6, 130.2, 131.1, 131.5, 134.5, 135.2, 136.2, 136.5, 137.4, 159.3, 168.8; IR (ATR): 1739, 1586, 1474, 1454, 1225 cm^{-1}. Anal. Calcd for $C_{27}H_{27}ClO_6S\cdot 1/4H_2O$: C, 62.42; H, 5.34. Found: C, 62.46; H, 5.32.

*4.2.5. 2-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-2-fluoromalonic acid diethyl ester (**8e**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 (t, 6H, J=7.2 Hz), 2.41–2.51 (m, 2H), 2.80–2.85 (m, 2H), 4.29 (q, 4H, J=7.2 Hz), 5.02 (s, 2H), 6.88–6.95 (m, 3H), 7.14 (s, 2H), 7.24 (t, 1H, J=7.9 Hz), 7.31–7.40 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.9, 14.0, 26.8, 26.9, 33.9, 34.1, 59.0, 62.4, 62.7, 70.1, 93.2, 95.2, 114.3, 117.6, 123.9, 127.5, 128.1, 128.6, 129.4, 130.2, 131.1, 131.4, 134.6, 135.6, 135.9, 136.5, 136.6, 159.3, 164.0, 165.8, 166.1; IR (ATR): 1748, 1586, 1474, 1185, 1016 cm^{-1}. Anal. Calcd for $C_{28}H_{28}ClFO_5S\cdot 1/2H_2O$: C, 62.27; H, 5.41. Found: C, 62.44; H, 5.55.

*4.2.6. 5-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-2,2-dimethyl-5-nitro[1,3]dioxane (**8f**)*

White solid; mp 101 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (s, 3H), 1.45 (s, 3H), 2.03–2.08 (m, 2H), 2.60–2.64 (m, 2H), 3.97 (d, 2H, J=13.0 Hz), 4.55 (d, 2H, J=13.0 Hz), 5.01 (s, 2H), 6.89–6.96 (m, 3H), 7.04–7.13 (m, 2H), 7.22–7.40 (m, 7H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.5, 26.1, 26.9, 33.9, 63.9, 70.1, 86.1, 99.2, 114.3, 117.8, 124.1, 127.5, 128.1, 128.6, 129.3, 130.2, 130.9, 131.2, 134.3, 135.6, 135.7, 136.3, 136.5, 159.3; IR (ATR): 1590, 1537, 822 cm^{-1}. Anal. Calcd for $C_{27}H_{28}ClNO_5S$: C, 63.09; H, 5.49; N, 2.72. Found: C, 62.79; H, 5.66; N, 2.58.

*4.2.7. 4-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-2-(diphenylmethyleneamino)butyric acid ethyl ester (**8g**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.27 (t, 3H, J=7.2 Hz), 2.16–2.24 (m, 2H), 2.61–2.76 (m, 2H), 4.09–4.23 (m, 3H), 5.01 (s, 2H), 6.86–6.94 (m, 3H), 7.06–7.11 (m, 2H), 7.15–7.43 (m, 15H), 7.68 (d, 2H, J=7.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2, 29.7, 33.3, 61.0, 64.9, 70.1, 114.0, 117.2, 123.5, 127.5, 127.8, 128.1, 128.5, 128.6, 128.7, 128.9, 129.6, 130.1, 130.4, 130.8, 131.6, 134.4, 134.6, 136.3, 136.4, 136.6, 138.3, 139.5, 159.3, 170.9, 172.0; IR (ATR): 1732, 1586, 1474, 1223, 1024, 694 cm^{-1}. Anal. Calcd for $C_{38}H_{34}ClNO_3S$: C, 73.59; H, 5.53; N, 2.26. Found: C, 73.24; H, 5.67; N, 2.29.

*4.2.8. 3-(2-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-ethyl)-3-(diphenylmethyleneamino)dihydrofuran-2(3H)-one (**8h**)*

White solid; mp 80–81 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.73–1.78 (m, 1H), 2.05–2.16 (m, 1H), 2.48–2.60 (m, 2H), 4.06–4.12 (m, 1H), 4.28–4.33 (m, 1H), 4.58–4.62 (m, 1H), 5.00 (s, 2H), 5.22–5.25 (m, 1H), 6.71 (d, 1H, J=7.9 Hz), 6.82–6.86 (m, 2H), 6.95–7.05 (m, 4H), 7.11–7.21 (m, 4H), 7.28–7.43 (m, 9H), 7.64 (d, 2H, J=7.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 39.2, 44.2, 46.9, 64.2, 65.5, 70.1, 113.6, 117.0, 123.1, 126.7, 127.2 (×2), 127.46, 127.52, 127.6, 128.1, 128.58, 128.62, 129.9, 130.0, 131.0, 134.5, 135.0, 136.4, 136.5, 138.4, 142.5, 146.5, 159.3, 179.3; IR (ATR): 1766, 1584, 1445, 1212, 1019, 697 cm^{-1}; HRMS (ESI): m/z calcd for $C_{38}H_{33}ClNO_3S$ $(M+H)^+$ 618.1870, found 618.1874. Anal. Calcd for $C_{38}H_{32}ClNO_3S\cdot 1/2H_2O\cdot 3/2HCl$: C, 66.93; H, 5.10; N, 2.05. Found: C, 66.62; H, 4.91; N, 2.04.

*4.3. 2-Chloro-4-[3-(hydroxy)phenylthio]benzaldehyde (**13**)*

A 500-mL, three-necked, round-bottomed flask equipped with an overhead mechanical stirrer with a large Teflon-coated blade, a rubber septum, and a Liebig reflux condenser fitted with an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with 2-chloro-4-fluoro-benzaldehyde (5.0 g, 31.7 mmol, 1 equiv), potassium carbonate (8.7 g, 63.3 mmol, 2.0 equiv), and 80 mL of anhydrous DMSO. The resulting mixture was stirred in an oil bath at 50 °C (internal temperature), then 3-hydroxybenzenthiol (4.0 g, 31.7 mmol, 1.0 equiv, in 30 mL of anhydrous THF) was added via syringe over 30 min. The addition proceeded at a rate that kept the internal temperature below 50 °C, usually taking 30 min. And the reaction mixture was stirred at 50 °C for 1.5 h, then the mixture was cooled to 0 °C in an ice-water bath, and 1 N HCl was added to the reaction mixture. The aqueous suspension was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from EtOAc/hexane, 15:85–30:70) to give 8.2 g (98%) of **13** as a yellow solid: mp 97–98 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.77 (s, 1H), 6.95 (dd, 1H, J=8.2, 2.0 Hz), 7.03 (dd, 1H, J=2.0, 2.0 Hz), 7.07–7.09 (m, 2H), 7.13 (d, 1H, J=2.0 Hz), 7.32 (dd, 1H, J=7.8, 7.8 Hz), 7.75 (d, 1H, J=8.2 Hz), 10.3 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 117.0, 121.2, 125.4, 126.7, 127.6, 129.4, 129.5, 131.1, 131.2, 138.6, 148.8, 156.8, 189.3; IR (ATR): 3269, 1656, 1573, 1437, 1250 cm^{-1}. Anal. Calcd for $C_{13}H_9ClO_2S$: C, 58.98; H, 3.43. Found: C, 59.11; H, 3.63.

*4.4. 4-[3-(Benzyloxy)phenylthio]-2-chlorobenzaldehyde (**14**)*

A 500-mL, three-necked, round-bottomed flask equipped with an overhead mechanical stirrer with a large Teflon-coated blade, a rubber septum, and a Liebig reflux condenser fitted with an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with compound **13** (4.7 g, 17.7 mmol, 1 equiv), potassium carbonate (5.9 g, 42.6 mmol, 2.4 equiv), and 80 mL of anhydrous MeCN. The resulting mixture was stirred at 0 °C in an ice-water bath, then benzyl bromide (3.6 g, 21.3 mmol, 1.4 equiv, in 20 mL of anhydrous MeCN) was added via syringe over 30 min. And the reaction mixture was stirred at 45 °C for 1.5 h, then the mixture was cooled to 0 °C in an ice-water bath, and 10 wt % aqueous citric acid was added to the reaction mixture. The aqueous suspension was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from EtOAc/hexane, 15:85–30:70) to give 6.2 g (98%) of **14** as a white solid: mp 48–49 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.07 (s, 2H), 7.05–7.07 (m, 2H), 7.11–7.13 (m, 3H), 7.33–7.42 (m, 6H), 7.74 (d, 1H, J=8.0 Hz), 10.35 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 70.2, 116.6, 120.6, 125.4, 127.0, 127.5, 127.7, 128.2, 128.7, 129.5, 129.6, 130.8, 131.2, 136.3, 138.5, 148.5, 159.6, 188.8; IR (ATR): 1684, 1575, 1242, 1019 cm^{-1}. Anal. Calcd for $C_{20}H_{15}ClO_2S$: C, 67.69; H, 4.26. Found: C, 67.64; H, 4.41.

*4.4.1. One-pot approach to synthesis of **14***

A 500-mL, three-necked, round-bottomed flask equipped with an overhead mechanical stirrer with a large Teflon-coated blade, a rubber septum, and a Liebig reflux condenser fitted with an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with 2-chloro-4-fluoro-benzaldehyde (5.3 g, 33.6 mmol, 1 equiv), potassium carbonate (9.3 g, 67.2 mmol, 2.0 equiv), and 70 mL of anhydrous DMSO. The resulting mixture was stirred in an oil bath at 50 °C (internal temperature), then 3-hydroxybenzenthiol (4.2 g, 33.6 mmol, 1.0 equiv, in 30 mL of anhydrous THF) was added via syringe over 30 min. The addition proceeded at a rate that kept the internal temperature below 50 °C, usually taking 30 min. And the reaction mixture was stirred at 50 °C for 1.5 h. After complete conversion was confirmed by TLC, the reaction mixture was stirred at 0 °C in an ice-water bath, then benzyl bromide (6.9 g, 40.3 mmol, 1.2 equiv, in 30 mL of anhydrous THF) was added via syringe over 30 min. And the reaction mixture was stirred at 45 °C for further 1.5 h, then the mixture was cooled to 0 °C in an ice-water bath, and 10 wt % aqueous citric acid was added to the reaction mixture. The aqueous suspension was extracted with EtOAc (3×50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from EtOAc/hexane, 15:85–30:70) to give 8.0 g (67%) of **14** as a white solid.

*4.5. 3-[3-(Benzyloxy)phenylthio]-6-vinyl-1-chlorobenzene (**9**)*

A 500-mL, three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stir bar, a rubber septum, and an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with compound **14** (5.0 g, 14.1 mmol,

1 equiv) and 140 mL of anhydrous THF. The resulting mixture was stirred at 0 °C in an ice-water bath, then 1 M $TMSCH_2MgCl$ (22 mL, 22 mmol, 1.5 equiv) was added via syringe over 30 min. And the reaction mixture was stirred at room temperature for 30 min, then the mixture was cooled to 0 °C in an ice-water bath, and 1 N HCl was added to the reaction mixture. The aqueous suspension was extracted with EtOAc (3×20 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The crude residue was dissolved in 140 mL of anhydrous THF. To the mixture was slowly added 1 M KO^{t}Bu in THF solution (31 mL, 31 mmol, 2.2 equiv) at 0 °C in an ice-water bath, and the reaction mixture was stirred at room temperature for 30 min, then the mixture was cooled to 0 °C in an ice-water bath, and 1 N HCl was added to the reaction mixture. The aqueous suspension was extracted with EtOAc (3×30 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from hexane only to EtOAc/hexane 30:70) to give 4.4 g (88%) of **9** as a yellow oil: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.11 (s, 2H), 5.46 (d, 1H, J=11.2 Hz), 5.91 (d, 1H, J=17.4 Hz), 6.94–7.05 (m, 4H), 7.20–7.22 (m, 1H), 7.30–7.42 (m, 7H), 7.69 (d, 1H, J=8.2 Hz); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 69.3, 114.9, 117.7, 118.0, 124.0, 127.4, 127.6, 127.8, 128.3, 128.6, 129.8, 130.7, 131.5, 132.5, 133.4, 134.1, 136.6, 136.8, 159.0; IR (ATR): 1583, 1472, 1222, 1024, 684 cm^{-1}. Anal. Calcd for $C_{21}H_{17}ClOS\cdot 1/8H_2O$: C, 71.02; H, 4.90. Found: C, 71.03; H, 4.96.

*4.5.1. Wittig approach to synthesis of **9***

A 1-L, three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stir bar, a rubber septum, and an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with methyltriphenylphosphonium bromide (15.6 g, 43.7 mmol) and 200 mL of anhydrous THF. The resulting mixture was stirred at 0 °C in an ice-water bath, then *n*-butyllithium (27 mL, 1.6 M in hexanes, 43.7 mmol) was added via syringe over 30 min. The orange mixture was stirred at room temperature for 1 h. To the reaction mixture was slowly added compound **14** (12.9 g, 36.4 mmol) in 100 mL of anhydrous THF at 0 °C in an ice-water bath. And the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was treated with saturated NH_4Cl solution (200 mL) and extracted with ethyl acetate (3×50 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation. The residue was purified by flash silica gel chromatography (gradient from hexane only to EtOAc/hexane 30:70) to give 11.3 g (88%) of **9** as a yellow oil.

*4.6. N-(3-{4-[3-(Benzyloxy)phenylthio]-2-chlorophenyl}-1,1-bis(hydroxymethyl)propyl)acetamide (**15**)*

A 500-mL, three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stir bar, a rubber septum, and an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with calcium chloride (2.9 g, 26.4 mmol, 2.5 equiv) and 20 mL of distilled water. To the resulting mixture was added compound **8a** (6.0 g, 10.5 mmol) in 100 mL of EtOH at 0 °C in an ice-water bath, then sodium borohydride (2.0 g, 52.7 mmol, 5 equiv) was added by potion-wise. And the reaction mixture was stirred at room temperature overnight. The mixture was cooled to 0 °C in an ice-water bath, and 1 N HCl was carefully added to the reaction mixture. The resulting mixture was directly concentrated by rotary evaporation. The residue was dissolved in $CHCl_3$, then washed with brine, dried over $MgSO_4$, filtered, and concentrated by rotary evaporation to give 4.9 g of **15** (95%) as a colorless oil which was sufficiently pure for use in the next reaction without further purification: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.88–1.93 (m, 2H), 2.04 (s, 3H), 2.70–2.74 (m, 2H), 3.64 (dd, 2H, J=11.3, 6.3 Hz), 3.73 (t, 2H, J=6.0 Hz), 3.89 (dd, 2H, J=11.3, 5.6 Hz), 5.02 (s, 2H), 6.02 (s, 1H), 6.87–6.94 (m, 3H), 7.13–7.18 (m, 2H), 7.23 (t, 1H, J=7.7 Hz), 7.31–7.41 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.1, 27.2, 33.3, 61.3, 65.7, 70.1, 114.1, 117.5, 123.7, 127.6, 128.1, 128.6, 129.8, 130.2, 131.0, 131.5, 134.1, 135.2, 136.1, 136.5, 138.0, 159.3, 171.7; IR (ATR): 3295, 1651, 1585, 1474, 1223, 1044, 1020, 684 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{26}H_{29}ClNO_4S$ $(M+H)^+$ 486.1506, found 486.1502.

*4.7. 2-Amino-2-(2-{4-[3-(benzyloxy)phenylthio]-2-chlorophenyl}ethyl)propane-1,3-diol hydrochloride (KRP-203, **2**)*

A 500-mL, three-necked, round-bottomed flask equipped with a Teflon-coated magnetic stir bar, a rubber septum, and an inert gas inlet tube that was attached to a nitrogen manifold. The flask was charged with compound **15** (5.7 g, 11.7 mmol), 20 mL of EtOH, and 20 mL of 6 M HCl. The reaction mixture was stirred at 80 °C for 60 min. The precipitate formed was collected by filtration to give 4.5 g of **2** (87%) as a white solid: mp 148–149 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.76–1.80 (m, 2H), 2.71–2.76 (m, 2H), 3.55 (d, 4H, J=4.8 Hz), 5.10 (s, 2H), 5.43 (t, 2H, J=4.8 Hz), 6.90–7.01 (m, 3H), 7.25 (dd, 1H, J=7.9, 1.2 Hz), 7.30–7.42 (m, 8H), 7.91 (br s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 25.9, 31.1, 60.0, 60.8, 69.2, 114.3, 116.9, 123.1, 127.6, 127.8, 128.3, 129.7, 130.6, 131.3, 133.4, 134.2, 135.1, 136.6, 138.3, 158.8; IR (ATR): 3243, 3033, 2940, 1587, 1521, 1243, 1072, 695 cm^{-1}. Anal. Calcd for $C_{24}H_{26}ClNO_3S\cdot HCl$: C, 60.00; H, 5.66; N, 2.92. Found: C, 59.72; H, 5.74; N, 2.90.

*4.8. Triethyl-2-(acetylamino)ethylene-1,1,2-tricarboxylate (**11**)*

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.29 (t, 3H, J=7.1 Hz), 1.31 (t, 3H, J=7.1 Hz), 1.34 (t, 3H, J=7.1 Hz), 2.20 (s, 3H), 4.23 (q, 2H, J=7.1 Hz), 4.28 (q, 2H, J=7.1 Hz), 4.33 (q, 2H, J=7.1 Hz), 10.94 (s, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.8, 12.0, 12.1, 22.0, 59.9, 60.0, 60.7, 103.6, 144.4, 160.4, 162.1, 164.8, 165.9; IR (ATR): 1721, 1602,

1236, 1196 cm^{-1}. Anal. Calcd for $C_{13}H_{19}NO_7 \cdot 1/10H_2O$: C, 51.20; H, 6.30; N, 4.59. Found: C, 51.15; H, 6.33; N, 4.79.

*4.9. 2,2-Dimethyl-5-nitro[1,3]dioxane (**7f**)*

A 50-mL round-bottomed flask equipped with a Teflon-coated magnetic stir bar and a nitrogen inlet. The flask was charged with 2,2-dimethyl-5-nitro[1,3]dioxan-5-ylmethanol[25–27] (1.14 g, 6.0 mmol) and 15 mL 10 wt % aqueous sodium hydroxide. The reaction mixture was heated to 60 °C for 1 h. The solution was cooled to 5 °C and at this temperature it was acidified to pH 5 with concentrated acetic acid. The precipitated solid was collected by filtration and dried to give 170 mg (18%) of **7f** as a pale brown solid: mp 60–61 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.23 (s, 3H), 1.42 (s, 3H), 4.28 (dd, 2H, J=13.0, 1.8 Hz), 4.38 (dd, 2H, J=13.0, 1.8 Hz), 4.63–4.64 (m, 1H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 18.9, 28.0, 59.2, 77.4, 98.0. Anal. Calcd for $C_6H_{11}NO_4 \cdot 1/10AcOH$: C, 43.11; H, 6.63; N, 8.38. Found: C, 43.41; H, 6.52; N, 8.29.

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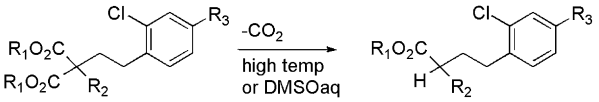
24. Also evaluated was the reaction temperature, which is crucial for limiting the formation of decarboxylation product. At above 100 °C or using the water content of DMSO, the major product was the corresponding decarboxylated compound.



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