(*Z*)-Heptadec-8-enylboronic acid: a potential lipase inhibitor

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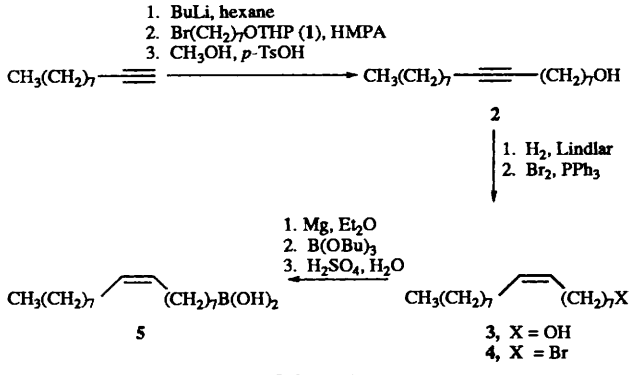
(*Z*)-Heptadec-8-enylboronic acid, a potential lipase inhibitor that is isosteric with oleic acid, and that is unexpectedly sensitive to aerial oxidation, has been prepared in 23% overall yield from dec-1-yne and 7-bromoheptanol. Disappointingly, the target compound proved inactive under conditions where lipase inhibition would have been expected.

Recently, there has been an explosion of interest in lipases,[1-3] particularly those found to be of value in synthetic organic chemistry, such as the enzymes from *Geotrichum candidum*[2] and *Candida rugosa*.[3] X-Ray structures provide unique insights into the mechanisms of action and specificities of enzymes and X-ray structural data are becoming increasingly available for synthetically useful lipases.[3a] In such studies, analyses of lipase enzyme-inhibitor (EI) complexes are particularly valuable, especially for the EI-complexes involving transition-state analogue inhibitors.[3a] While achieving specific inhibition of triglyceride lipases is difficult,[4] it is known that simple alkyl- and aryl-boronic acids that are able to act as transition-state inhibitors of serine proteases, to which class lipases belong, also inhibit lipoprotein lipase,[5] porcine pancreatic lipase[6] and human bile-salt-dependent lipase.[7] These results, together with the fact that the natural substrates of lipases are glycerol triesters of fatty acids such as oleic acid, suggested that (*Z*)-heptadec-8-enylboronic acid **5**, the boronic acid isostere of oleic acid. would be an effective specific transition-state analogue inhibitor of the synthetically useful lipases from *G. candidum* and *C. rugosa*. The boronic acid **5** has now been prepared as outlined in Scheme 1.



Scheme 1

The coupling of the THP-protected bromoheptanol **1** with 1-lithiodecyne afforded heptadec-8-yn-1-ol **2** in 80% yield. This was then hydrogenated to give the *cis*-alcohol **3**, followed by treatment with bromine–triphenylphosphine, to give (*Z*)-1-bromoheptadec-8-ene **4** in 94% overall yield from **2**. While the reactions involved in the transformation of **4** into the target compound **5** all proceeded smoothly, the instability of the product, and the resultant difficult-to-remove by-products, reduced the isolable yield of pure product to 32% for the **4** ⟶ **5** steps. Despite this, an overall yield of 23% was achieved for the Scheme 1 sequence.

Boronic acids with long alkyl chains, such as lauryl, myristyl and stearyl, are generally more stable than those with shorter butyl or hexyl chains.[8] However, surprisingly, when pure, (*Z*)-heptadec-8-enylboronic acid **5** was found to be quite unstable, even when stored under an inert atmosphere. Although esterification with bis(2-hydroxyethyl)amine usually confers additional stability and handling benefits on unstable boronic acids,[9] this approach did not bestow any such advantages when applied to **5**. Purification of the target inhibitor **5** was eventually achieved by careful recrystallisation from acetone at −78 °C. Unfortunately, this recrystallised material was also unstable, and decomposed rapidly even while being dried *in vacuo*. The main product of this decomposition was found to be (*Z*)-heptadec-8-en-1-ol **3**, which is apparently formed by oxidation[10] of **5** when even trace amounts of oxygen are present. The (*Z*)-heptadec-8-enylboronic acid **5** was eventually stored at 0 °C as a 0.5 mol dm^{-3} solution in dry, oxygen-free, diethyl ether, under which conditions very little decomposition was observed even after six months. The boronic acid **5** is then recoverable by careful removal of the diethyl ether by rotary evaporation at room temperature, immediately prior to use.

Disappointingly, when **5** was evaluated as an inhibitor of the lipases from *G. candidum* and *C. rugosa*, it proved impossible to obtain EI-complexes crystallographically suitable for X-ray studies.[11] Evaluating inhibitors of lipases is not straightforward because of the difficulties in ensuring access into the 'lidded' active sites, and inhibition is highly influenced by the natures of the solvents used to open-up access to the active sites. However, no inhibition of *G. candidum* or *C. rugosa* lipases by **5** could be detected at [I] to [E] ratios of up to 30 000 to 1. using diethyl ether, acetone or 2-methylpentane-2,4-diol (the preferred *G. lipase* X-ray-crystallisation solvent), or using polydimethylsiloxane.[11] In contrast, irreversible sulfonyl chloride inhibitors[3d] in diethyl ether did effect 50% inhibition of both lipases at [I]:[E] levels >1000:1.[11] Thus the current evidence is that, despite its resemblance to the putative transition state, the boronic acid **5** is not an effective competitive inhibitor of lipases. However, a possibility that cannot yet be excluded is that the negative results may derive from the extreme sensitivity of the boronic acid **5** to oxygen during the attempted kinetics and X-ray crystal soakings, in both aqueous and organic solvent conditions, and also to its poor aqueous solubility. Any one, or combination, of these factors could preclude reaching inhibitory concentrations of **5**. This unanticipated instability of the oleic acid side chain–boronic acid combination represents a stumbling block that those interested in lipase-inhibitor studies should be aware of. We did not evaluate the saturated analogue of **5** and are now exploring alternative strategies to boronic acid inhibitors of lipases.

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Experimental

General methods

Unless otherwise stated, all reactions were performed under nitrogen using oven-dried glassware. TLC plates were analysed by spraying with 5% aqueous $KMnO_4$. Preparative flash column chromatography was performed using silica gel 60 (40–63 μm), supplied by Toronto Research Chemicals Inc. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Kugelrohr boiling points are uncorrected. IR spectra were determined on KBr pellets (for solids) and films (for liquids) with a Nicolet 5DX FTIR spectrophotometer. NMR (^1H, ^{13}C) spectra were recorded on a Gemini 200 (at 200 MHz and 50 MHz, respectively) spectrometer. J Values are given in Hz. Mass spectra were measured on Bell and Howell 21-490 (low resolution) or AEI MS3074 (high resolution) instruments.

Preparation of (*Z*)-heptadec-8-enylboronic acid 5

Several crystals of toluene-*p*-sulfonic acid (*p*-TsOH) were added to a stirred mixture of 7-bromoheptan-1-ol (5 g, 26 mmol) and dihydropyran (4.66 cm^3, 52 mmol) cooled to −10 °C, and the solution then allowed to warm to room temperature (20 °C). The reaction mixture was stirred for 3 h and then K_2CO_3 was added, and stirring continued for a further 1 h. The reaction mixture was then filtered, the solvent removed by rotary evaporation, and the residue Kugelrohr-distilled to give 1-bromo-7-(tetrahydropyran-2-yloxy)heptane **1** (6.9 g, 96%), bp 105–108 °C/0.2 mmHg; ν(neat)/cm^{-1} 2934, 2878, 1464, 1259, 1201, 1034, 1024, 869 and 531; $\delta_H(CDCl_3)$ 1.34–1.93 (16 H, m), 3.30–3.54 (4 H, m), 3.67–3.91 (2 H, m) and 4.55 (1 H, m); $\delta_C(CDCl_3)$ 19.60, 25.40, 25.97, 28.00, 28.49, 29.53, 30.67, 32.64, 33.81, 62.23, 67.39 and 98.74.

To a slurry of 1-lithiodec-1-yne, prepared at −10 °C from dec-1-yne (3.04 g, 22 mmol) in dry tetrahydrofuran (THF) and butyllithium (1.6 mol dm^{-3} solution in hexane; 15 cm^3, 24 mmol), was added 1-bromo-7-(tetrahydropyran-2-yloxy)heptane **1** (6.7 g, 24 mmol) in hexamethylphosphoramide (HMPA) (30 cm^3) while maintaining the temperature below 0 °C.[12] The mixture was then allowed to warm to room temperature and stirred for an additional 10 h. The reaction mixture was worked up by pouring into ice–water (200 cm^3) and extracting with hexanes (3 × 50 cm^3). The hexane extracts were washed with water (30 cm^3), dried ($MgSO_4$), and concentrated on a rotary evaporator. Methanol (100 cm^3) and *p*-TsOH (10 mg) were then added and the mixture refluxed for 3 h. After cooling to room temperature, K_2CO_3 (5 g) was added, the solution stirred for 1 h, filtered and the filtrate rotary evaporated. Kugelrohr distillation afforded heptadec-8-yn-1-ol **2** (4.45 g, 80%), bp 120–124 °C/0.25 mmHg; ν(neat)/cm^{-1}) 3334br, 2928, 2856, 1465, 1434 and 723; $\delta_H(CDCl_3)$ 0.85 (3 H, t, J 6.4), 1.24–1.57 (23 H, m), 2.05–2.11 (4 H, m) and 3.61 (2 H, t, J 6.4); $\delta_C(CDCl_3)$ 14.04, 18.68, 22.61, 25.60, 28.74, 28.82, 28.90, 29.02, 29.08, 29.11, 29.17, 31.79, 32.67, 62.87, 80.03 and 80.26.

A mixture of heptadec-8-yn-1-ol **2** (4.28 g, 17 mmol), Lindlar[13] catalyst (150 mg) and freshly distilled quinoline (300 mm^3) in cyclohexene (60 cm^3), was stirred under H_2 for 3 h (1 equiv. H_2 uptake). The mixture was filtered, washed successively with 5% aqueous HCl (3 × 20 cm^3), 5% aqueous $NaHCO_3$ (20 cm^3), and H_2O (20 cm^3), and then dried ($MgSO_4$) and filtered. Rotary evaporation of the filtrate followed by Kugelrohr distillation gave (*Z*)-heptadec-8-en-1-ol **3** (4.18 g, 97%), bp 123–126 °C/0.25 mmHg; ν(neat)/cm^{-1} 3334br, 3302, 2929, 2853, 1651, 1465, 1057 and 722; $\delta_H(CDCl_3)$ 0.85 (3 H, t, J 6.2), 1.18–1.40 (21 H, m), 1.49–1.58 (2 H, m), 1.97–2.05 (4 H, m), 3.61 (2 H, m) and 5.32 (2 H, m); $\delta_C(CDCl_3)$ 14.08, 22.66, 25.70, 27.15, 27.19, 29.22, 29.30d, 29.50, 29.59, 29.67, 29.74, 31.88, 32.75, 62.98, 129.72 and 129.95.

To a solution of bromine–triphenylphosphine[14] [from Ph_3P (4.46 g, 17 mmol) and Br_2 (2.72 g, 17 mmol)] in dry CH_2Cl_2 (60 cm^3) at 0 °C was added, in one portion, (*Z*)-heptadec-8-en-1-ol **3** (4.0 g, 16 mmol) in dichloromethane (10 cm^3) and the mixture stirred for 1 h at 0 °C. The reaction mixture was then washed with 10% aqueous K_2CO_3 (50 cm^3), and the organic layer washed further with water (2 × 25 cm^3). After drying ($MgSO_4$) the solvent was removed by rotary evaporation, then hexanes (30 cm^3) were added to the residue. The precipitated triphenylphosphine oxide was filtered off and washed thoroughly with hexanes (5 × 15 cm^3). All of the hexane solutions were combined, rotary evaporated, and the residue chromatographed on a short silica gel column [diethyl ether–hexanes (3:10) elution, R_f 0.22] and finally Kugelrohr-distilled to yield (*Z*)-1-bromoheptadec-8-ene **4** (4.84 g, 97%), bp 121–125 °C/0.25 mmHg; ν(neat)/cm^{-1} 3006, 2956, 2925, 2853, 1651, 1464, 1440 and 723; $\delta_H(CDCl_3)$ 0.84 (3 H, t, J 6.3), 1.12–1.45 (20 H, m), 1.77–1.91 (2 H, m), 1.93–2.06 (4 H, m), 3.44 (2 H, t, J 6.5) and 5.33 (2 H, m); $\delta_C(CDCl_3)$ 14.10, 22.68, 27.11, 27.21, 28.14, 28.66, 29.05, 29.32d, 29.52, 29.61, 29.76, 31.90, 32.81, 33.91, 129.62 and 130.03.

Mg powder (0.58 g, 24 mmol, just covered with dry diethyl ether) was mechanically activated[15] under argon for 2 days, and a solution of (*Z*)-1-bromoheptadec-8-ene **4** (4.66 g, 14.6 mmol) in dry diethyl ether (20 cm^3) then added dropwise at room temperature. The resulting Grignard reagent (formation complete after 3.5 h) was added during 10 min to tributyl borate (3.93 cm^3, 14.6 mmol) in dry diethyl ether (50 cm^3), while maintaining the reaction temperature below −60 °C. After all the Grignard reagent had been added, the resulting white suspension was allowed to warm slowly to room temperature, then cooled to 0 °C and 10% aqueous H_2SO_4 (20 cm^3) added. The diethyl ether layer was removed and the aqueous phase extracted with diethyl ether (3 × 25 cm^3). The combined organic phases were dried ($MgSO_4$), rotary evaporated, and the residue chromatographed on silica gel [diethyl ether–hexanes (1:1) elution, R_f 0.34]. The solvents were removed on a rotary evaporator at 20 °C (no heat!) and the residue crystallised from acetone at −78 °C. The crystalline product (which decomposed immediately when drying under vacuum was attempted) was dried in a Schlenk tube in a dry N_2 stream to give (*Z*)-heptadec-8-enylboronic acid **5** (1.32 g, 32%), mp 56–58 °C; ν(KBr)/cm^{-1} 3465, 3325, 3001, 2958, 2924, 2846, 1468, 1391 and 805; $\delta_H(CDCl_3)$ 0.78–0.91 (5 H, m), 1.24–1.38 (22 H, m), 1.94–2.02 (4 H, m), 4.23 (variable intensity, s, OH) and 5.32 (2 H, m); $\delta_C(CDCl_3)$ 14.50, 16.02 (br), 23.10, 23.74, 24.75, 27.64, 29.66, 29.75, 29.95, 30.20, 32.33, 32.70, 32.81, 33.03, 130.13 and 130.34 (Found: M^+, 792.7930. Calc. for $C_{51}H_{99}B_3O_3$ (trimeric anhydride): M, 792.7873).

The crystalline **5** obtained above was very unstable and was therefore stored as a 0.5 mol dm^{-3} solution in oxygen-free dry diethyl ether. When solid material was needed, the ethereal solution was carefully rotary evaporated at 20 °C (no heat!) to recover pure **5** in a microcrystalline form that was used immediately.

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