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Rapid Microwave-Assisted Syntheses of Derivatives of HIV-1 Entry Inhibitors

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Abstract: The direct amidation of esters with 4-amino-2,2,6,6-tetramethylpiperidine was achieved by computer-controlled microwave irradiation in toluene. The microwave protocol allowed the new amides to be prepared in three hours rather than the three days required by traditional thermal methods.

Key words: microwave-assisted synthesis, HIV, amidation, CCR5, gp120

Knowledge of both the mechanism of action of the HIV virus and the structures of the many protein–protein interactions that are involved in HIV entry into cells is central to developing possible treatments and cures for those infected. The entry of HIV-1 into a host is mediated by the interaction between the viral envelope glycoprotein gp120 and the host cell receptor CD4. This entry triggers a cascade of conformational changes in the viral envelope glycoprotein resulting in the exposure of the co-receptor (CXCR4 or CCR5) binding site on gp120.[1–4] The binding of gp120 to the coreceptor leads to the destabilization of the gp120/gp41 complex.[5–8] As a result, gp41 undergoes conformational change and exposure of the hydrophobic fusion peptide, which inserts into the target cell membrane and initiates the HIV-1 entry process (Figure 1).[9–12] Therefore, the binding between gp120 and CD4 is a key step for HIV-1 entry into cells and has been suggested as a potential novel target for anti-HIV-1 therapy.[9,13–17]

The X-ray structure of an HIV-1 envelope glycoprotein gp120 core domain complexed to a two-domain fragment (D1D2) of the cellular receptor CD4 and to the Fab fragment of the human neutralizing antibody 17b (gp120-CD4-17b)[14,18] and a recently published structure of an unliganded simian immunodeficiency virus (SIV) gp120 core, indicated distinct conformational differences in the unliganded and the CD4-bound structures.[19,20] These structures also revealed a narrow cavity in the gp120 core structure near the CD4 binding site, and that cavity has been suggested as the putative binding site of BMS-378806, a potent entry inhibitor reported by a group at Bristol-Meyers Squib.[21,22] In order to identify other small molecule compounds that block gp120-CD4 interactions, we initiated a screening program of commercially available chemical libraries. Recently, the two *N*-phenyl-*N*′-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide analogues **1** and **2** were identified as novel human immunodeficiency virus type 1 (HIV-1) entry inhibitors that block the gp120-CD4 interaction (Figure 2).[23] These small organic molecules showed potent cell fusion and virus-cell fusion inhibitory activity at low micromolecular levels.[23] Inspired by these initial findings, we set out to develop a new protocol that would allow the rapid synthesis of a variety of derivatives of these new entry inhibitors.

Our synthetic strategy to prepare derivatives of **1** and **2** was influenced by the large number of substituted anilines

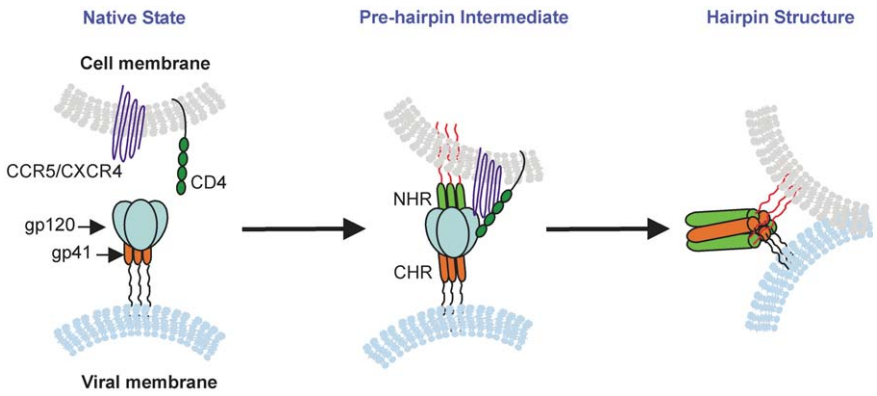

Figure 1 Schematic diagram of the HIV-1 entry process.

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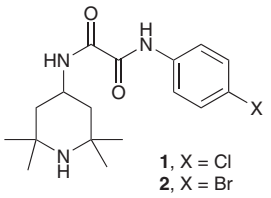


Figure 2

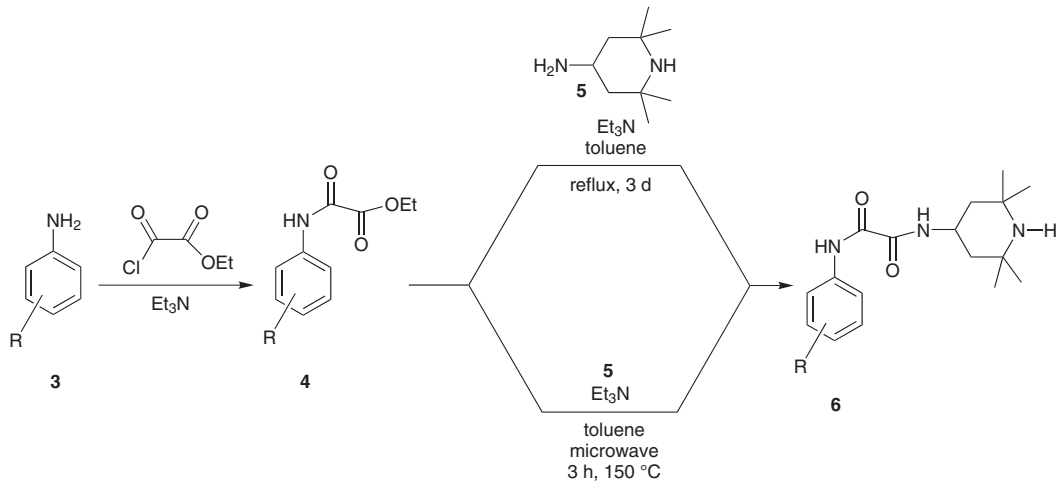
that are commercially available. An attractive route is the addition of a substituted aniline to ethyl chlorooxoacetate in the presence of base (Scheme 1)[24] to provide access to a large number of building blocks of the general structure **4**, which could then be further derivatized. Despite the poor nucleophilicity of electron deficient anilines, we found that their additions to ethyl chlorooxoacetate proceeded smoothly and the yields of the resulting esters **4** were generally in the range 80–90%. Figure 3 shows the variety of esters that have been prepared by this method. With this mild procedure, even aminopyridines could be condensed leading to the nitrogen-rich products **7**, **22**, and **25**. The silyl-protected ethers **27** and **28** were also prepared, potentially providing access to two new hydroxyl-containing derivatives.

With esters of the general structure **4** in hand, we then examined whether esters of type **4** could be directly converted to amides **6** without having to convert the ester functionality to an acid chloride or whether peptide coupling procedures would be necessary using the carboxylic acid derivative. Unfortunately, we found that the thermal amidation reactions with 4-amino-2,2,6,6-tetramethylpiperidine (**5**) to afford **6** (Scheme 1) proceeded quite slowly, requiring three days at reflux in toluene solution. These thermal reactions were clearly not suitable to rapidly prepare derivatives of **1** and **2**.

We therefore turned our attention to the use of microwave technology to aid in the amidation reactions with **5**. Microwave-assisted chemical synthesis has enjoyed explosive growth in recent years as developments in the microwave protocols provide rapid, 'green', and predictable chemical functionalizations.[25–36] The dramatic rate enhancements that have also been observed using microwave technology[28] make it a promising method for the current study. Indeed, repeating the amidation reactions with **5** under microwave conditions produced **6** in only three hours in yields that were adequate for biochemical inhibition studies. The reactions were not run to completion, but rather optimized for rapid throughput, hence moderate yields were typical. Figure 4 shows the new entry inhibitor analogues that were prepared via the microwave procedure.

Compounds **1** and **2** were also re-synthesized by the microwave protocol, and X-ray quality crystals of **2** were grown in the hope of providing a low-energy model of this class of active HIV-1 entry inhibitors for future docking studies. The ORTEP diagram of **2** is shown in Figure 5.[37] Interestingly, the carbonyl groups of the amide linkages were found to lie out of the plane of the aromatic ring. This structural feature can perhaps be rationalized by the intermolecular hydrogen bonding of the amide groups observed in the packing diagram of **2** (Figure 6). The hydrogen of the alkyl amide portion of **2** forms a close contact (2.310 Å) with the aryl amide oxygen O2 of another molecule and no hydrogen bonding was observed for the aryl amide hydrogen of N1. The solid-state interactions provide insight into some hydrogen-bonding capabilities of **2**, but because of the intermolecular interactions, it appears that computational methods are best suited to providing a low-energy model appropriate for solution-phase simulations.

The microwave-assisted chemical synthesis of novel human immunodeficiency virus type 1 (HIV-1) entry inhibitors that block the gp120-CD4 interaction has been achieved. The microwave methods allow the derivatives to be synthesized in a fraction of the time compared to normal thermal methods, and also circumvent the need to convert esters **4** to acid chlorides or carboxylic acids before further derivatization.



Scheme 1

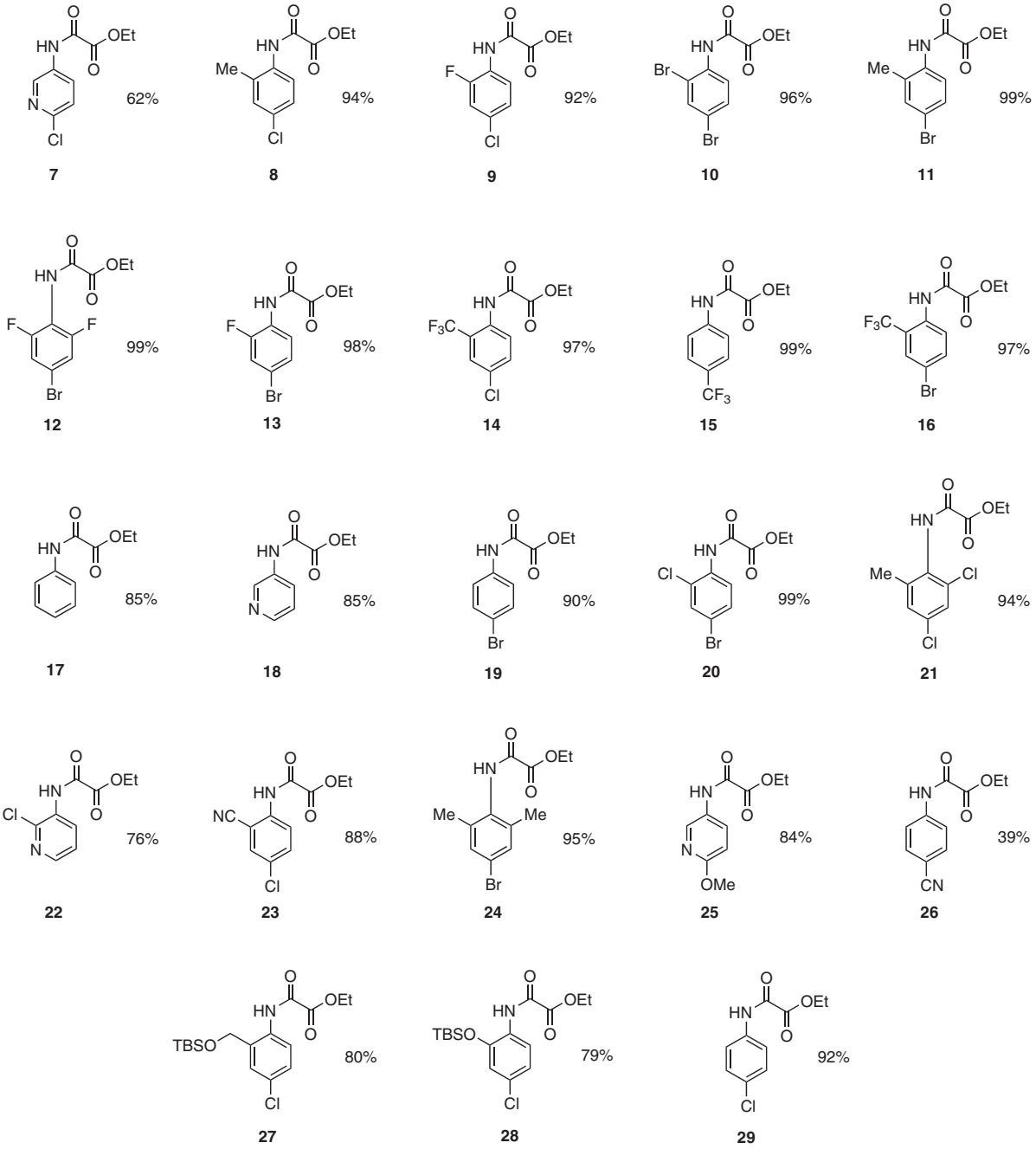


Figure 3

Unless otherwise noted, all reactions were carried out under a nitrogen atmosphere. All commercially available compounds were purchased from Aldrich Chemical Company, GFS Chemicals, or Alfa Aesar Organics and used as received unless otherwise specified. THF, Et_2O, toluene, and CH_2Cl_2 were distilled over a drying agent prior to use. All ^1H and ^{13}C NMR spectra were obtained on a JEOL 270 MHz or a Bruker 300 MHz (for ^1H) instrument (67 MHz or 75 MHz for ^{13}C, respectively). LRMS were conducted on an Agilent system. Mps were acquired on a Mel-Temp II melting point apparatus by Laboratory Devices. Microwave reactions were carried out using a CEM Discovery System with an Explorer auto-sampler attachment.

Esters 7-28; Typical Procedure

Ethyl chlorooxoacetate (620 μL, 5.58 mmol) was added dropwise to a cooled (0 °C) mixture of 4-chloro-2-fluoroaniline (5.58 mmol) and Et_3N (660 μL, 5.58 mmol) in THF (50 mL). The reaction mixture was allowed to warm slowly to r.t. and stirred for 12 h. The mixture was then filtered to remove the ammonium salts, and the filtrate was washed with HCl (2 N; 1 × 50 mL). The organic layer was then dried over $MgSO_4$, filtered, and concentrated under vacuum yielding *N*-(2-fluoro-4-chlorophenyl)oxalamic acid ethyl ester (**9**), an off-white crystalline solid, in 92% yield.

^1H NMR (300 MHz, $CDCl_3$): δ = 1.40 (t, *J* = 7.1 Hz, 3 H), 4.40 (q, *J* = 7.1 Hz, 2 H), 7.14 (dd, *J* = 2.2, 8.8 Hz, 2 H), 8.33 (t, *J* = 8.5 Hz, 1 H), 9.06 (br s, 1 H).

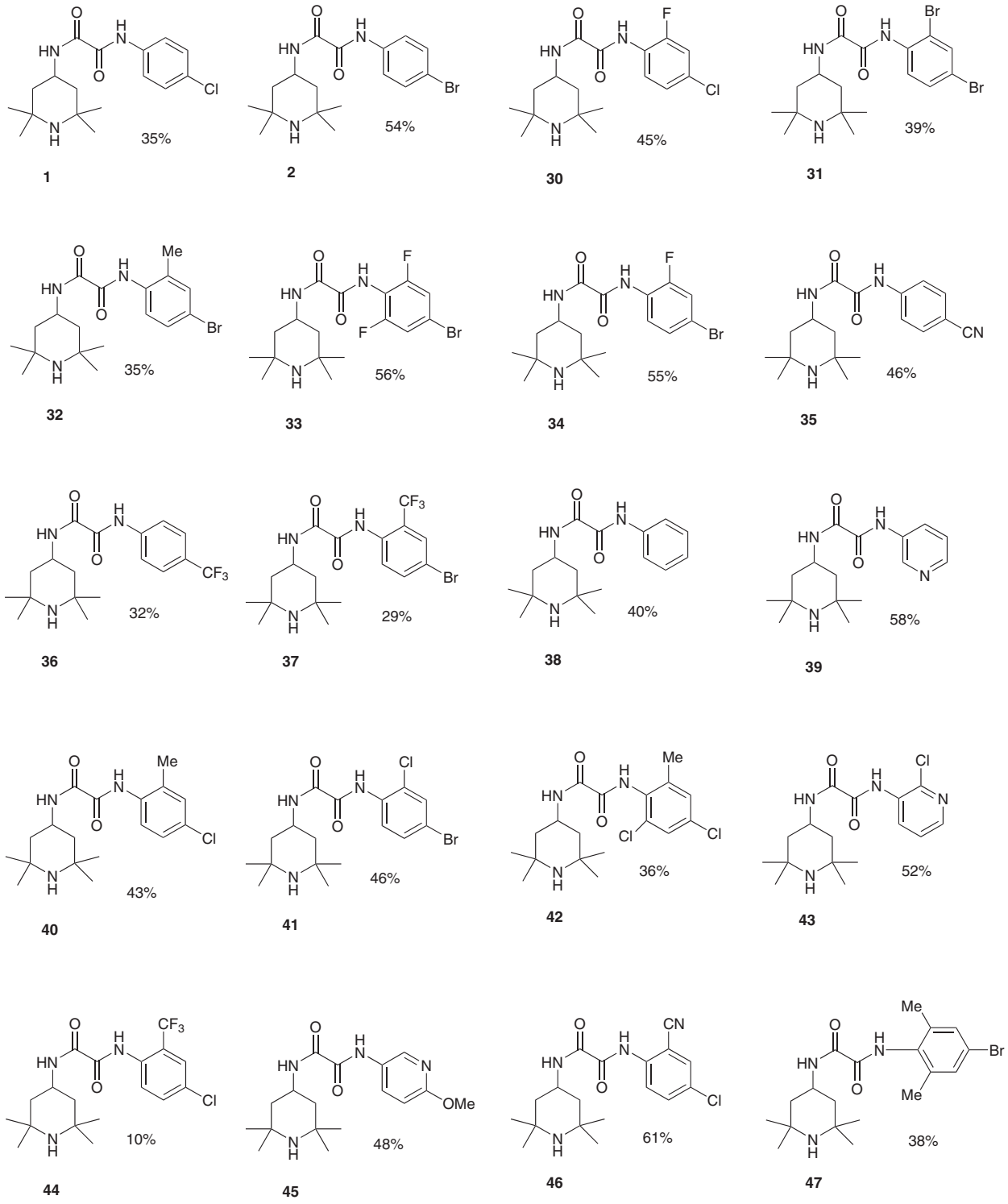


Figure 4

^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.0, 64.0, 116.0 (d, *J* = 22.2 Hz), 122.0, 123.7 (d, *J* = 10.3 Hz), 125.1, 130.4 (d, *J* = 9.8 Hz), 150.6, 153.9, 160.2.

LRMS: *m*/*z* calcd for $C_{10}H_9ClFNO_3$ (M^+): 245.6; found: (M^+) 245.

Microwave-Assisted Amide Bond Formation; Typical Procedure

4-Amino-2,2,6,6-tetramethylpiperidine (80 μL, 0.46 mmol) was added to a solution of *N*-(2-cyano-4-chlorophenyl)oxalamic acid ethyl ester (**23**; 0.46 mmol) and Et_3N (108 μL, 0.92 mmol) in toluene (5 mL). The reaction vial was sealed with a crimp-top and placed in a microwave where the temperature was raised to 150 °C in 10 min and held at that temperature for 3 h. Then HCl (2 N; 10

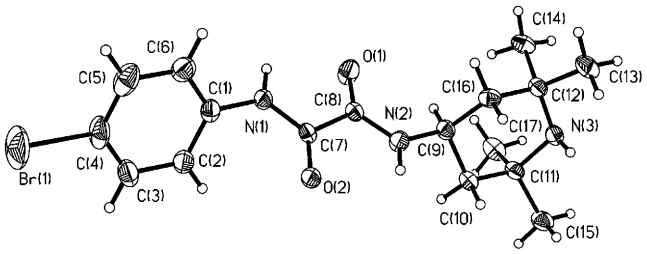


Figure 5 ORTEP diagram of **2**. Ellipsoids shown at the 40% level. Selected bond lengths (Å): N(1)–C(7) 1.340(6); N(1)–C(1) 1.418(6); C(7)–C(8) 1.532(6); N(2)–C(8) 1.319(6); N(2)–C(9) 1.462(6); O(1)–C(8) 1.228(6); O(2)–C(7) 1.222(5). Selected bond angles (°): C(7)–N(1)–C(1) 124.3(4); C(8)–N(2)–C(9) 123.4(4); N(1)–C(7)–C(8) 112.7(4); O(1)–C(8)–N(2) 124.9(4); O(2)–C(7)–N(1) 125.5(4); O(2)–C(7)–C(8) 121.8(4).

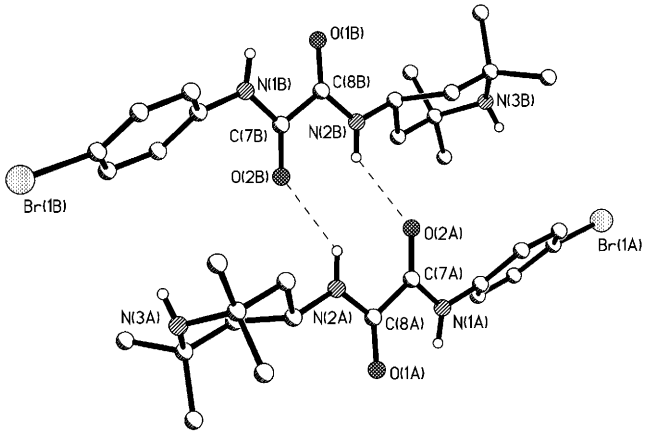


Figure 6 Packing diagram of **2**. All hydrogens except those attached to nitrogen have been omitted for clarity.

mL) was added to the reaction mixture and extracted with EtOAc (3 × 10 mL). Following the initial extraction, the remaining aqueous layer was basified with 2 N NaOH and re-extracted with CH_2Cl_2 (3 × 10 mL). The CH_2Cl_2 layer was dried over $MgSO_4$, filtered, and concentrated under vacuum providing *N*-(2-cyano-4-chlorophenyl)-*N*′-(2,2,6,6-tetramethylpiperidin-4-yl)oxalamide (**46**), as a yellow crystalline solid in 61% yield.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.13 (t, *J* = 12.4 Hz, 2 H), 1.16 (s, 6 H), 1.27 (s, 6 H), 1.91 (dd, *J* = 3.3, 12.4 Hz, 2 H), 4.28 (m, 1 H), 7.56 (m, 2 H), 8.31 (d, *J* = 8.8 Hz, 1 H).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.3, 34.6, 43.9, 44.3, 51.3, 104.9, 114.4, 122.2, 130.5, 132.1, 134.3, 137.4, 157.9, 158.1.

LRMS: *m*/*z* calcd for $C_{18}H_{23}ClN_4O_2$ (M^+): 362.9; found: (M^+) 362.

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(37) Crystallographic data (excluding structure factors) for compound **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 286628. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax:+44 (1223)336033 or e-mail: deposit@ccdc.cam.ac.uk].