

# Structure-activity relationships of a small-molecule inhibitor of the PDZ domain of PICK1†

Anders Bach,<sup>a</sup> Nicolai Stuhr-Hansen,<sup>a</sup> Thor S. Thorsen,<sup>b</sup> Nicolai Bork,<sup>c</sup> Irina S. Moreira,<sup>d</sup> Karla Frydenvang,<sup>a</sup> Shahrokh Padrah,<sup>a</sup> S. Brøgger Christensen,<sup>a</sup> Kenneth L. Madsen,<sup>b</sup> Harel Weinstein,<sup>d</sup> Ulrik Gether\*<sup>b</sup> and Kristian Strømgaard\*<sup>a</sup>

<sup>a</sup>*Department of Medicinal Chemistry, University of Copenhagen, Universitetsparken 2, DK-2100 Copenhagen, Denmark. E-mail: krst@farma.ku.dk; Fax: (+45) 3533 6041; Tel (+45) 3533 6114.*

<sup>b</sup>*Department of Neuroscience and Pharmacology, Panum Institute, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen, Denmark. E-mail: gether@sund.ku.dk; Fax (+45) 3532 7610; Tel: (+45) 3532 7548.*

<sup>c</sup>*Risø National Laboratory for Sustainable Energy, Technical University of Denmark, Frederiksborgvej 399, DK-4000 Roskilde, Denmark*

<sup>d</sup>*Department of Physiology and Biophysics, Weill Medical College of Cornell University, New York, NY 10021, USA.*

## ELECTRONIC SUPPLEMENTARY INFORMATION

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## CHEMISTRY

### General procedures

All reagents were obtained from commercial suppliers and used without further purification. Proton ( $^1\text{H}$ ) NMR spectra were recorded on a Varian spectrometer, Mercury Plus (300 MHz) and carbon ( $^{13}\text{C}$ ) NMR spectra were recorded on a Varian spectrometers, Gemini 2000 (75 MHz). Chemical shifts ( $\delta$ ) are reported relative to tetramethylsilane ( $\text{Me}_4\text{Si}$ ). The following abbreviations are used for the proton spectra multiplicities: s, singlet; br s, broad singlet; d, doublet; dd, double doublet, triplet; q, quartet; sep, septet m, multiplet. Coupling constants ( $J$ ) are reported in hertz (Hz). Elemental analyses were performed by Mr. J. Theiner, Department of Physical Chemistry, University of Vienna, Austria. Purities for compounds **32-35** were determined by analytical HPLC on an Agilent 1100 system with a C18 reverse phase column (Zorbax 300 SB-C18 column, 4.6×150 mm), flow rate of 1 mL/min, and a linear gradient of a binary solvent system of water/acetonitrile/TFA (A: 95/5/0.1 and B: 5/95/0.1). Melting points were determined on a MPA100 OptiMelt melting point apparatus. TLC analysis was performed on silica gel F<sub>254</sub> (Merck) and detection was carried out by UV light and staining with potassium permanganate. Flash column chromatography was performed on silica gel 60F with solvent of HPLC grade.

**Condensation reaction between 2-cyanoacetic acid and carbamates (Compounds 2, 15-18, 20, 23, 24). General Procedure.** Cyanoacetic acid (1 equiv, 50 mmol), carbamate (1.1 equiv, 55 mmol) and phosphorous oxychloride (0.52 equiv, 26 mmol) were dissolved in toluene (10 mL) and dry DMF (1.2 mL) was added under nitrogen. The reaction mixture was heated for 1.5 hours at 70 °C. The mixture was cooled to room temperature and poured into ice (50 g). The resultant suspension was transferred into a separation funnel, the organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with brine (20 mL) and dried over magnesium sulfate, filtrated and concentrated in vacuum. The crude product was purified by flash chromatography (heptane/ethyl acetate, 8:2), unless otherwise noted.

**Knoevenagel condensation reaction between cyanoacetylcarbamates and benzaldehydes (Compounds 1 and 25-49). General Procedure.** Aldehyde (1 equiv, 1.71 mmol) and cyanoacetylcarbamate (1 equiv, 1.71 mmol) were dissolved in dry DMF (2 mL) under nitrogen, after which catalytic amounts of piperidine (0.1 equiv, 0.17 mmol) and acetic acid (0.1 equiv, 0.17 mmol) were added. The reaction mixture was stirred at room temperature for 2 hours, unless otherwise noted.

The reaction mixture was concentrated in vacuum and the crude product purified by recrystallization from absolute ethanol, unless otherwise noted.

**(E)-Ethyl 2-cyano-3-(3,4-dichlorophenyl)acryloylcarbamate (1).** Yield: 43%; white crystals; Mp 175-178 °C (from toluene); (Found: C, 49.86; H, 3.22; N, 8.95. Calc. for C<sub>13</sub>H<sub>10</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C, 49.89; H, 2.96; N, 8.83%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.24 (3 H, t, *J* 7.1), 4.18 (2 H, q, *J* 7.1), 7.46 (1 H, d, *J* 8.5), 7.72 (1 H, dd, *J* 8.5 and 2.2), 7.95 (1 H, d, *J* 2.2), 8.02 (1 H, s), 9.69 (1 H, s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 63.3, 104.8, 115.6, 129.6, 131.0, 131.5, 132.7, 134.1, 138.3, 149.8, 153.2, 157.2.

**Ethyl 2-cyanoacetylcarbamate (2).** Yield: 82%; white crystals; Mp 166-167 °C (from heptane/ethyl acetate); (Found: C, 46.12; H, 5.08; N, 17.98. Calc. for C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub>: C, 46.15; H, 5.16; N, 17.94%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.20 (3 H, t, *J* 7.1), 3.83 (2 H, s), 4.11 (2 H, q, *J* 7.1), 10.33 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.2, 27.8, 62.4, 113.5, 152.2, 163.6.

**Ethyl 2-cyano-3-(3,4-dichlorophenyl)propanoylcarbamate (3).** Palladium (5% on charcoal, 15 mg, 0.007 mmol) was added to a solution of (*E*)-ethyl 2-cyano-3-(3,4-dichlorophenyl)acryloylcarbamate (**1**) (0.062 g, 0.2 mmol) in ethyl acetate (2 mL) in a test tube which was sealed with a rubber septum and mounted with a hydrogen balloon. The black slurry was stirred at room temperature for 12 hours and filtered through celite (1 g). Evaporation of the solvent and recrystallization from ethanol (0.5 mL) gave **3** (0.048 g, 76%) as white flakes: Mp 129-130 °C (from ethanol); (Found: C, 49.78; H, 3.83; N, 8.78. Calc. for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 49.54; H, 3.84; N, 8.90%);  $\delta_{\text{H}}$  (300 MHz, DMSO-*d*<sub>6</sub>) 1.23 (3 H, t, *J* 7.1), 3.05-3.10 (1 H, m), 3.21-3.26 (1 H, m), 4.16 (2 H, q, *J* 7.1), 4.36-4.48 (1 H, m), 7.31-7.33 (1 H, m), 7.61-7.63 (2 H, m), 11.16 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, DMSO-*d*<sub>6</sub>) 14.2, 33.7, 41.0, 61.8, 117.0, 129.7, 130.1, 130.8, 131.1, 131.3, 137.7, 151.2, 165.0.

**(E)-3,4-Dichlorocinnamoyl chloride (4).** 3,4-Dichlorocinnamic acid (8.69 g, 40 mmol) was added to ice-cooled thionyl chloride (70 mL) in one portion under stirring. After stirring at room temperature for 15 minutes the reaction was heated at reflux for 2 hours. Thionyl chloride was evaporated and bulb-to-bulb distillation (120 Pa, 180 °C) gave **4** (6.91 g, 73%) as a colourless liquid which solidified by standing at room temperature:  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 6.63 (1 H, d, *J* 15.7), 7.38 (1 H, dd, *J* 2.2 and 8.2), 7.50 (1 H, d, *J* 8.2), 7.64 (1 H, d, *J* 2.2), 7.72 (1 H, d, *J* 15.7);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 124.3, 127.9, 130.6, 131.4, 133.1, 133.9, 136.3, 147.6, 165.8.

**(E)-Ethyl 3-(3,4-dichlorophenyl)acryloylcarbamate (5) and ethyl (E)-3,4-dichlorocinnamate (6).**

Sodium hydride (60% in mineral oil, 0.12 g, 3 mmol) was added to ethanol-ice cooled dry DMF (15 mL) in one portion under nitrogen. Urethane (0.29 g, 3.3 mmol) was added in one portion and stirring was maintained for 1 hour at room temperature and cooled in an ethanol-ice bath while 3,4-dichlorocinnamoyl chloride (**4**) (0.71 g, 3 mmol) was added in one portion. After stirring at 1 hour at room temperature, the light-yellow reaction mixture was poured into ice (150 g) and a yellow precipitate (0.8 g) was filtered off. Separation was performed by flash chromatography on silica gel (20 g) by means of ethyl acetate/heptane (1:2) affording the by-product ethyl (E)-3,4-dichlorocinnamate (**6**; 0.31 g, 42%) as white plates: Mp: 51-52 °C (from ethyl acetate/heptane); (Found: C, 53.99; H, 4.11. Calc. for C<sub>11</sub>H<sub>10</sub>Cl<sub>2</sub>O<sub>2</sub>: C, 53.90; H, 4.11%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.34 (3 H, t, *J* 7.1), 4.27 (2 H, q, *J* 7.1), 6.41 (1 H, d, *J* 16.0), 7.34 (1 H, dd, *J* 2.0 and 8.2), 7.45 (1 H, d, *J* 8.2), 7.56 (1 H, d, *J* 16.0), 7.60 (1 H, d, *J* 2.0);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 60.9, 120.3, 127.1, 129.7, 131.0, 133.4, 134.3, 134.7, 141.9, 166.4; *m/z* (EI) 212 (100), 178 (44). The desired acryloylcarbamate **5** was eluted as the second product, which after evaporation of the solvent and drying in a vacuum oven (100 Pa, 60 °C, overnight) was obtained (0.12 g, 14%) as white crystals: Mp 187-189 °C (from ethyl acetate/heptane); (Found: C, 50.44; H, 3.79; N, 4.75. Calc. for C<sub>12</sub>H<sub>11</sub>Cl<sub>2</sub>NO<sub>3</sub>: C, 50.02; H, 3.85; N, 4.86%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.34 (3 H, t, *J* 7.1), 4.27 (3 H, q, *J* 7.1), 7.41 (1 H, dd, *J* 2.0 and 8.4), 7.47 (1 H, d, *J* 8.4), 8.50 (1 H, br s), 7.60 (1 H, d, *J* 15.6), 7.68 (1 H, d, *J* 2.0), 7.73 (1 H, d, *J* 15.6);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 62.7, 95.6, 119.8, 127.7, 128.1, 130.0, 131.0, 134.7, 143.5, 151.4, 165.9.

**N-butyl-2-cyanoacetamide (7).** Ethyl 2-cyanoacetylcarbamate (**2**) (0.74 g, 2 mmol) was added to butylamine (1.0 M solution in methanol, 20 mL, 20 mmol). The clear colourless solution was stirred for 1 hour at room temperature and evaporated on celite. Separation was performed by flash chromatography on silica gel (20 g) by means of heptane/ethyl acetate (2:1). Evaporation of the solvent gave **7** (0.51 g, 91%) as white crystals: Mp 71-72 °C (from heptane/ethyl acetate; lit. mp<sup>1</sup> 151-151.6 °C); (Found: C, 59.87; H, 8.57; N, 20.12. Calc. for C<sub>7</sub>H<sub>12</sub>N<sub>2</sub>O: C, 59.98; H, 8.63; N, 19.98%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>); 0.91-0.96 (3 H, m), 1.32-1.42 (2 H, m), 1.48-1.58 (2 H, m), 3.26-3.32 (2 H, m), 3.39 (3 H, s), 6.42 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.9, 20.2, 26.1, 31.4, 40.3, 115.0, 161.0; *m/z* (EI) 139 (M<sup>+</sup>, 1), 125 (6), 111 (27), 97 (100).

**(E)-N-Butyl-2-cyano-3-(3,4-dichlorophenyl)acrylamide (8).** Acetic acid (0.006 mL, 0.1 mmol) and piperidine (0.008 g, 0.1 mmol) was added to a solution of *N*-butyl-2-cyanoacetamide (**7**) (0.14 g, 1

mmol) and 3,4-dichlorobenzaldehyde (0.18 g, 1 mmol) in DMF (5 mL). Stirring was maintained for 24 hours at room temperature. The resulting orange solution was poured into water (200 mL) and extracted with ethyl acetate (3 × 40 mL). The pooled extracts were washed with water (10 mL) and evaporated on celite. Separation on silica was performed using ethyl acetate/heptane (1:2). After evaporation of the solvent, recrystallization from ethanol gave **8** (0.14 g, 47%) as white crystals: Mp 145-146 °C (from ethanol); (Found: C, 56.36; H, 5.66; N, 4.72. Calc. for C<sub>14</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>2</sub>O: C, 56.58; H, 5.55; N, 4.75%);  $\delta_{\text{H}}$  (300 MHz, DMSO-*d*<sub>6</sub>) 0.87-0.91 (3 H, m), 1.28-1.34 (2 H, m), 1.47-1.49 (2 H, m), 3.19-3.24 (2 H, m), 7.85 (1 H, d, *J* 8.4), 7.92 (1 H, dd, *J* 1.6 and 8.4), 8.14 (1 H, s), 8.16 (1 H, d, *J* 1.6), 8.47 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, DMSO-*d*<sub>6</sub>) 13.7, 19.6, 31.0, 40.2, 108.6, 115.9, 129.5, 131.5, 131.7, 131.9, 132.7, 134.5, 147.8, 160.3; *m/z* (EI) 296 (M<sup>+</sup>, 19), 281 (4), 267 (7), 253 (23), 239 (29), 224 (100).

**2-(Hydroxy(3,4-dichlorophenyl)methyl)acrylonitrile (9)**. A solution of 3,4-dichlorobenzaldehyde (8.75 g, 50 mmol), acrylonitrile (7.98 g, 150 mmol) and diazabicyclooctane (DABCO) (6.17 g, 55 mmol) in dioxane-water (1:1, 120 mL) was stirred at room temperature for 4 hours. The yellow solution was poured into brine (250 mL) and extracted with diethyl ether (3 × 40 mL). The pooled ethereal phases were washed with water (30 mL), dried with magnesium sulfate, and concentrated. The residual yellow oil was distilled by bulb-to-bulb distillation (100 Pa, 220 °C) affording **9** (7.75 g, 68%) as a colourless oil: (Found: C, 52.62; H, 3.00; N, 6.18. Calc. for C<sub>10</sub>H<sub>7</sub>Cl<sub>2</sub>NO: C, 52.66; H, 3.09; N, 6.14%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 3.23 (1 H, br s), 5.23 (1 H, s), 6.04 (1 H, d, *J* 1.3), 6.09 (1 H, d, *J* 1.3), 7.20 (1 H, dd, *J* 2.2 and 8.2), 7.45 (1 H, d, *J* 8.2), 7.46 (1 H, d, *J* 2.2);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 73.2, 116.9, 125.8, 126.2, 128.3, 131.2, 131.3, 133.3, 133.4, 139.7; *m/z* (EI) 227 (M<sup>+</sup>, 24), 192 (5), 175 (100).

**(E)-2-(Bromomethyl)-3-(3,4-dichlorophenyl)acrylonitrile (10) and 2-(bromo(3,4-dichlorophenyl)methyl)acrylonitrile (11)**. Phosphorous tribromide (1.03 mL, 11 mmol) in diethyl ether (15 mL) was added dropwise over 20 minutes to a vigorously stirred ice-ethanol cooled solution of 2-(hydroxy(3,4-dichlorophenyl)methyl)acrylonitrile (**9**) (2.28 g, 10 mmol) in diethyl ether (150 mL). After stirring at room temperature for 2 hours the clear colourless solution was poured into ice (100 g). After separation, the aqueous phase was extracted with diethyl ether (2 × 40 mL) and the combined organic phases were washed with water (10 mL), dried with magnesium sulfate, and evaporated. Filtration through silica gel (15 g) by means of ethyl acetate/heptane (1:2), evaporation of

the solvent and drying in a vacuum oven (100 Pa, 70 °C, overnight) gave a 1:1 mixture (estimated from GCMS) of **10** and **11** (2.01 g, 69%) as a white crystalline mass: (Found: C, 41.49; H, 2.08; N, 4.75. Calc. for C<sub>10</sub>H<sub>6</sub>BrCl<sub>2</sub>N: C, 41.28; H, 2.08; N, 4.81%).

**(Z)-2-(aminomethyl)-3-(3,4-dichlorophenyl)acrylonitrile (12) and 2-(Amino(3,4-dichlorophenyl)methyl)acrylonitrile (13).** The 1:1 mixture (1.74 g, 6 mmol) of (*E*)-2-(bromomethyl)-3-(3,4-dichlorophenyl)acrylonitrile (**10**) and 2-(bromo(3,4-dichlorophenyl)methyl)acrylonitrile (**11**) was added to ammonia (2.0 M solution in methanol, 40 mL, 80 mmol) in one portion as a solid. After stirring for 2 hours at room temperature the light-yellow reaction mixture was poured into water and extracted with diethyl ether (3 × 40 mL). The combined ethereal extracts were dried over sodium sulfate, filtered and evaporated. Separation was performed by flash chromatography on silica gel (25 g) by means of ethyl acetate/heptane (1:2). First fraction contained **13** (0.89 g, 65%) as a colourless liquid: (Found: C, 52.81; H, 3.62; N, 12.45. Calc. for C<sub>10</sub>H<sub>8</sub>Cl<sub>2</sub>N<sub>2</sub>: C, 52.89; H, 3.55; N, 12.34%); δ<sub>H</sub> (300 MHz, CDCl<sub>3</sub>); 4.67 (1 H, s), 6.01 (1 H, s), 6.08 (1 H, s), 7.24 (1 H, dd, *J* 2.2 and 8.2), 7.45 (1 H, d, *J* 2.2), 7.50 (1 H, d, *J* 8.2); δ<sub>C</sub> (75 MHz, CDCl<sub>3</sub>) 57.8, 117.1, 126.3, 127.5, 128.9, 129.9, 130.9, 132.5, 133.1, 140.7; *m/z* (EI) 226 (M<sup>+</sup>, 3), 210 (1), 191 (4), 174 (100). Continuous elution gave a second fraction, which after evaporation and drying in a vacuum oven (100 Pa, 40 °C, overnight) gave the desired acrylonitrile **12** (0.26 g, 19%) as a white crystalline mass. Analytically pure crystals were prepared by recrystallization from heptane affording white crystals: Mp 64-65 °C (from heptane); (Found: C, 52.75; H, 3.48; N, 12.23. Calc. for C<sub>10</sub>H<sub>8</sub>Cl<sub>2</sub>N<sub>2</sub>: C, 52.89; H, 3.55; N, 12.34%); δ<sub>H</sub> (300 MHz, CDCl<sub>3</sub>); 1.49 (2 H, br s), 3.64 (2 H, d, *J* 1.4), 7.04 (1 H, s), 7.50 (1 H, d, *J* 8.5), 7.65 (1 H, dd, *J* 1.9 and 8.5), 7.76 (1 H, d, *J* 1.9); δ<sub>C</sub> (75 MHz, CDCl<sub>3</sub>) 46.8, 115.6, 117.7, 126.1, 127.6, 130.7, 131.0, 133.4, 134.0, 139.5; *m/z* (EI) 226 (M<sup>+</sup>, 100), 210 (12), 198 (33).

**(Z)-Ethyl 2-cyano-3-(3,4-dichlorophenyl)allylcarbamate (14).** Ethyl chloroformate (0.052 mL, 0.55 mmol) in THF (0.3 mL) was added dropwise over 5 minutes to an ice-cooled solution of (*Z*)-2-(aminomethyl)-3-(3,4-dichlorophenyl)acrylonitrile (**12**) (0.11 g, 0.5 mmol) in triethylamine/THF (1:1, 3 mL) under nitrogen. The light-yellow reaction mixture was stirred at room temperature for 2 hours and methanol (10 mL) was added. After evaporation on celite the product was separated on silica gel (15 g) by means of ethyl acetate/heptane (1:2). Evaporation of the solvent and drying in a vacuum oven (100 Pa, 60 °C, overnight) provided **14** (0.076 g, 64%) as white crystals. Recrystallization from

toluene gave analytically pure white needles: Mp 116-117 °C (from toluene); (Found: C, 52.36; H, 3.99; N, 9.24. Calc. for C<sub>13</sub>H<sub>12</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>2</sub>: C, 52.19; H, 4.04; N, 9.36%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.27 (3 H, t, *J* 7.1), 4.07-4.09 (1 H, m), 4.16 (3 H, q, *J* 7.1), 5.19 (1 H, br s), 7.06 (1 H, s), 7.50 (1 H, d, *J* 8.4), 7.65 (1 H, dd, *J* 2.2 and 8.4), 7.76 (1 H, d, *J* 2.2);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.8, 45.7, 62.4, 111.1, 117.9, 128.3, 131.5, 131.6, 133.5, 133.9, 135.3, 142.6, 177.5; *m/z* (EI) 298 (M<sup>+</sup>, 48), 269 (12), 251 (18), 225 (100).

**Isopropyl 2-cyanoacetylcarbamate (15).** Yield: 67%; white crystals; Mp 119-120 °C (from ethyl acetate); (Found: C, 49.31; H, 5.86; N, 16.49. Calc. for C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>: C, 49.41; H, 5.92; N, 16.46%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.32 (6 H, d, *J* 6.2), 4.05 (2 H, s), 5.1 (1 H, sep), 7.73 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 21.7, 27.7, 70.5, 113.5, 151.5, 163.4.

**Butyl 2-cyanoacetylcarbamate (16).** Yield: 46%; pale yellow crystals; Mp 121-22 °C (from ethanol); (Found: C, 52.25; H, 6.57; N, 15.26. Calc. for C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 52.17; H, 6.57; N, 15.21%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 0.93-0.97 (3 H, m), 1.37-1.43 (2 H, m), 1.63-1.69 (2 H, m), 4.04 (2 H, s), 4.19-4.22 (2 H, m), 7.95 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.7, 19.0, 27.8, 30.6, 67.3, 113.1, 151.8, 163.9.

**Ethyl 2-cyanoacetyl(methyl)carbamate (17).**<sup>2</sup> Yield: 88%; white crystals; Mp 35-37 °C (from heptane/ethyl acetate); (Found: C, 49.16; H, 5.85; N, 16.38. Calc. for C<sub>7</sub>H<sub>10</sub>N<sub>2</sub>O<sub>3</sub>: C, 49.41; H, 5.92; N, 16.46%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.32 (3 H, t, *J* 7.2), 3.20 (3 H, s), 4.09 (2 H, s), 4.25 (2 H, q, *J* 7.0);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.2, 29.9, 31.6, 63.9, 113.9, 153.9, 164.8.

**Ethyl 2-cyanoacetyl(ethyl)carbamate (18).** Yield: 93%; colorless oil; (Found: C, 51.60; H, 6.65; N, 14.55. Calc. for C<sub>8</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 52.17; H, 6.57; N, 15.21%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.17 (3 H, t, *J* 7.0 Hz), 1.36 (3 H, t, *J* 7.1), 3.81 (2 H, q, *J* 7.0), 4.09 (2 H, s), 4.30 (2 H, q, *J* 7.1);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.8, 14.3, 30.2, 40.4, 63.9, 113.9, 153.9, 164.4.

**Carbamic acid adamantan-1-yl ester (19).** Prepared according to literature.<sup>3</sup> Yield: 87%; white crystals; Mp 166-167 °C (from heptane/ethyl acetate); (Found: C, 67.42; H, 8.79; N, 7.15. Calc. for C<sub>11</sub>H<sub>17</sub>NO<sub>2</sub>: C, 67.66; H, 8.78; N, 7.17%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.65 (6 H, s), 2.10 (6 H, s), 2.15 (3 H, br s), 4.69 (2 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 31.0, 36.3, 41.6, 79.6, 156.1.

**Cyanoacetylcarbamic acid adamantan-1-yl ester (20).** Yield: 26%; white crystals; Mp 96-98 °C (from heptane);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.68 (6 H, t,  $J$  3.1), 2.14 (6 H, d,  $J$  3.2), 3.38 (2 H, s), 2.21 (4 H, br s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 26.3, 31.1, 36.1, 41.2, 84.6, 113.6, 150.0, 161.4, 177.2.

**Ethyl *N*-butylcarbamate (21).**<sup>4</sup> Ethyl chloroformate (9.53 mL, 100 mmol) was added to cooled vigorously stirred butylamine (60 mL) over a 10 minutes period under nitrogen. Stirring was maintained for 1 hour at room temperature and the reaction mixture was filtered through aluminum oxide (activated basic, 20 g) by ethyl acetate. The solvent was evaporated into a thick white slurry and successive bulb-to-bulb distillation (100 Pa, 135 °C) gave **21** (6.17g, 42%) as a colourless liquid: (Found: C, 57.98; H, 10.59; N, 9.54. Calc. for  $\text{C}_7\text{H}_{15}\text{NO}_2$ : C, 57.90; H, 10.41; N, 9.65%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 0.92 (3 H, t,  $J$  7.3), 1.24 (3 H, t,  $J$  7.1), 1.32-1.39 (2 H, m), 1.44-1.50 (2 H, m), 3.15-3.19 (2 H, m), 4.11 (2 H, q,  $J$  7.3), 4.62 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 13.9, 14.8, 20.1, 32.2, 40.7, 60.6, 156.7.

**Ethyl *N*-benzylcarbamate (22).**<sup>4</sup> Ethyl chloroformate (4.77 mL, 50 mmol) was added over 10 minutes to an ice-cold mixture of benzylamine (8.04 g, 75 mmol), triethyl amine (50 mL) and THF (50 mL) under nitrogen. Stirring was maintained for 2 hours at room temperature and the colourless reaction mixture was poured into ice (200 g) and extracted with ethyl acetate (4 × 40 mL). After drying over sodium sulfate the solvent was evaporated. Bulb-to-bulb distillation (100 Pa, 135 °C) gave **22** (5.81 g, 43%) as a colourless liquid: (Found: C, 67.20; H, 7.35; N, 7.88. Calc. for  $\text{C}_{10}\text{H}_{13}\text{NO}_2$ : C, 67.02; H, 7.31; N, 7.82%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.24 (3 H, t,  $J$  7.1), 4.11-4.17 (2 H, q,  $J$  7.1), 4.35 (2 H, d,  $J$  5.9), 5.06 (1 H, br s), 7.24-7.35 (5 H, m);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 14.8, 45.0, 60.9, 127.3, 127.4, 128.5, 138.6, 156.7.<sup>5</sup>

**Ethyl butyl(2-cyanoacetyl)carbamate (23).** Yield: 66%; colourless oil; (Found: C, 56.30; H, 7.65; N, 13.14. Calc. for  $\text{C}_{10}\text{H}_{16}\text{N}_2\text{O}_3$ : C, 56.59; H, 7.60; N, 13.20%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 0.94 (3 H, t,  $J$  7.2), 1.26-1.40 (5 H, m), 1.49-1.59 (2 H, m), 3.73-3.78 (2 H, m), 4.09 (2 H, s), 4.31 (2 H, q,  $J$  7.1);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 13.9, 14.4, 20.1, 30.2, 30.6, 44.9, 63.9, 113.9, 154.1, 164.6.

**Ethyl benzyl(2-cyanoacetyl)carbamate (24).** Yield: 52%; white crystals; Mp 60-61 °C (from heptane/ethyl acetate); (Found: C, 63.34; H, 5.62; N, 11.36. Calc. for  $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_3$ : C, 63.40; H, 5.73; N, 11.38%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.32 (3 H, t,  $J$  7.1), 4.14 (2 H, s), 4.29 (2 H, q,  $J$  7.1), 4.96 (2 H, s),



7.27-7.35 (5 H, m);  $\delta_C$  (75 MHz,  $CDCl_3$ ) 14.2, 30.2, 47.9, 64.2, 113.8, 127.9, 128.2, 128.6, 136.4, 153.8, 164.8.

**(E)-Ethyl 2-cyano-3-(naphthalen-2-yl)acryloylcarbamate (25).** Yield: 66%; pale yellow crystals; Mp 165-168 °C (from ethanol); (Found: C, 68.96; H, 4.73; N, 9.28. Calc. for  $C_{17}H_{14}N_2O_3$ : C, 69.38; H, 4.79; N, 9.52%);  $\delta_H$  (300 MHz,  $CDCl_3$ ) 1.37 (3 H, t,  $J$  7.1), 4.33 (2 H, q,  $J$  7.1), 7.56-7.66 (2 H, m), 7.87-7.95 (3 H, m), 8.14 (1 H, dd,  $J$  8.6 and 1.8), 8.27 (1 H, br s), 8.36 (1 H, s), 8.54 (1 H, s);  $\delta_C$  (75 MHz,  $CDCl_3$ ) 14.1, 62.8, 102.3, 116.4, 124.8, 127.3, 127.8, 128.7, 129.2, 129.3, 129.4, 132.6, 134.8, 135.5, 149.8, 156.0, 157.8.

**(E)-Ethyl 3-(anthracen-9-yl)-2-cyanoacryloylcarbamate (26).** Yield: 62%; pale orange crystals; Mp 167-169 °C (from toluene); (Found: C, 72.99; H, 4.91; N, 7.45. Calc. for  $C_{21}H_{16}N_2O_3$ : C, 73.24; H, 4.68; N, 8.13%);  $\delta_H$  (300 MHz,  $CDCl_3$ ): 1.37 (3 H, t,  $J$  7.1), 4.36 (2 H, q,  $J$  7.1), 7.50-7.62 (4 H, m), 7.94 (2 H, d,  $J$  8.7), 8.06 (2 H, d,  $J$  8.2), 8.27 (1 H, br s), 8.58 (1 H, s), 9.46 (1 H, s);  $\delta_C$  (75 MHz,  $CDCl_3$ ) 14.2, 62.3, 114.1, 115.5, 124.4, 125.0, 125.4, 126.9, 128.6, 128.8, 130.2, 130.6, 151.0, 153.4, 159.6.

**(E)-Ethyl 3-(biphenyl-4-yl)-2-cyanoacryloylcarbamate (27).** Yield: 58%; light yellow crystals; Mp 170-171 °C (from ethanol); (Found: C, 71.21; H, 4.95; N, 8.67. Calc. for  $C_{19}H_{16}N_2O_3$ : C, 71.24; H, 5.03; N, 8.74%);  $\delta_H$  (300 MHz,  $CDCl_3$ ): 1.37 (3 H, t,  $J$  7.1), 4.30-4.35 (2 H, q,  $J$  7.1), 7.43-7.51 (3 H, m), 7.63-7.66 (2 H, m), 7.75 (2 H, d,  $J$  10.4), 8.06 (2 H, d,  $J$  8.4), 8.23 (1 H, br s), 8.43 (1 H, s);  $\delta_C$  (75 MHz,  $CDCl_3$ ) 14.1, 62.8, 102.0, 116.4, 127.1, 127.8, 128.6, 129.0, 130.0, 131.8, 139.1, 146.5, 149.8, 155.5, 157.8.

**(E)-Isopropyl 2-cyano-3-(3,4-dichlorophenyl)acryloylcarbamate (28).** Yield: 31%; white crystals; Mp 188-190 °C (from ethanol); (Found: C, 51.32; H, 3.55; N, 8.48. Calc. for  $C_{14}H_{12}Cl_2N_2O_3$ : C, 51.40; H, 3.70; N, 8.56%);  $\delta_H$  (300 MHz,  $CDCl_3$ ) 1.35 (6 H, d,  $J$  6.2), 5.10 (1 H, sep), 7.61 (1 H, d,  $J$  8.4), 7.85 (1 H, dd,  $J$  8.4 and 2.0), 8.02 (1 H, d,  $J$  2.0), 8.12 (1 H, br s), 8.30 (1 H, s);  $\delta_C$  (75 MHz,  $CDCl_3$ ) 21.6, 71.2, 104.7, 115.4, 129.3, 130.8, 131.3, 132.5, 133.9, 138.0, 149.1, 152.8, 157.1.

**(E)-Isopropyl 2-cyano-3-(naphthalen-2-yl)acryloylcarbamate (29).** Yield: 31%; yellow crystals; Mp 176-177 °C (from ethanol); (Found: C, 69.04; H, 4.98; N, 8.84. Calc. for  $C_{18}H_{16}N_2O_3$ : C, 70.12; H,

5.23; N, 9.09%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.35 (6 H, d,  $J$  6.4), 5.11 (1 H, sep), 7.56-7.66 (2 H, m), 7.87-7.96 (3 H, m), 8.14 (1 H, dd,  $J$  8.6 and 1.7), 8.22 (1 H, br s), 8.36 (1 H, s), 8.54 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 21.6, 70.9, 102.4, 116.4, 124.8, 127.2, 127.8, 128.7, 129.2, 129.3, 129.4, 132.6, 134.8, 135.5, 155.8, 157.9.

**(E)-Isopropyl 3-(anthracen-9-yl)-2-cyanoacryloylcarbamate (30).** Purified by flash chromatography (heptane/ethyl acetate, 90:10) followed by recrystallization from toluene. Yield: 20%; pale orange crystals; Mp 176-178 °C (from toluene); (Found: C, 72.37; H, 4.83; N, 7.58. Calc. for  $\text{C}_{22}\text{H}_{18}\text{N}_2\text{O}_3$ : C, 73.73; H, 5.06; N, 7.82%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.36 (6 H, d,  $J$  6.2), 5.14 (1 H, sep), 7.53-7.63 (4 H, m), 7.94 (2 H, d,  $J$  8.2), 8.07 (2 H, d,  $J$  8.2), 8.12 (1 H, br s), 8.59 (1 H, s), 9.48 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 21.4, 69.7, 113.7, 115.9, 124.2, 124.8, 125.1, 126.4, 128.2, 128.4, 129.6, 130.2, 150.3, 152.3, 159.7.

**(E)-Isopropyl 3-(biphenyl-4-yl)-2-cyanoacryloylcarbamate (31).** Yield: 44%; pale yellow crystals; Mp 186-188 °C (from ethanol); (Found: C, 71.71; H, 5.28; N, 8.30. Calc. for  $\text{C}_{20}\text{H}_{18}\text{N}_2\text{O}_3$ : C, 71.84; H, 5.43; N, 8.38%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.35 (6 H, d), 5.10 (1 H, sep), 7.42-7.51 (3 H, m), 7.63-7.65 (2 H, m), 7.75 (2 H, d,  $J$  8.6), 8.06 (2 H, d,  $J$  8.8), 8.18 (1 H, br s), 8.43 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 21.6, 70.9, 102.1, 116.4, 127.1, 127.8, 128.6, 129.0, 123.0, 131.8, 139.1, 146.4, 149.3, 155.4, 157.8.

**(E)-Adamantan-1-yl 2-cyano-3-(3,4-dichlorophenyl)acryloylcarbamate (32).** Yield: 58%; pale yellow crystals; Mp 150-151 °C (from ethanol); Pure by analytical HPLC (96%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.71 (6 H, s), 2.23 (10 H, s), 7.56 (1 H, d,  $J$  8.4), 7.87 (1 H, dd,  $J$  8.5 and 1.9), 7.96 (1 H, d,  $J$  2.1), 8.02 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 31.1, 36.2, 41.3, 84.6, 106.8, 115.2, 129.2, 131.3, 131.4, 132.6, 133.8, 137.2, 150.9, 160.1.

**(E)-Adamantan-1-yl 2-cyano-3-(naphthalen-2-yl)acryloylcarbamate (33).** Yield: 74%; pale yellow crystals; Mp 148-149 ° (from ethanol); Pure by analytical HPLC (97%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.73 (6 H, s), 2.27 (10 H, s), 7.53-7.64 (2 H, m), 7.86-7.95 (3 H, m), 8.17 (1 H, dd,  $J$  8.7 and 1.8), 8.29 (1 H, s), 8.34 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 30.8, 35.9, 41.1, 83.7, 104.4, 115.8, 127.0, 127.7, 128.7, 128.9, 129.1, 129.2, 132.7, 133.6, 135.1, 153.7, 160.9.

**(E)-Adamantan-1-yl 3-(anthracen-9-yl)-2-cyanoacryloylcarbamate (34).** Yield: 50%; yellow crystals; Mp 195-197 °C (from heptane); Pure by analytical HPLC (95%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.77 (6 H, s), 2.35 (7 H, s), 2.31 (3 H, br s), 7.50-7.63 (4 H, m), 7.97 (2 H, d,  $J$  8.3), 8.05 (2 H, d,  $J$  8.4), 8.56 (1 H, s), 9.19 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 31.2, 36.3, 41.4, 84.6, 114.5, 114.6, 124.8, 125.6, 125.8, 127.3, 129.0, 129.3, 130.6, 131.1, 153.1, 154.3, 159.9.

**(E)-Adamantan-1-yl 3-(biphenyl-4-yl)-2-cyanoacryloylcarbamate (35).** Yield: 68%; white crystals; Mp 180-181 °C (from ethanol); Pure by analytical HPLC (95%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.73 (6 H, s), 2.26 (10 H, s), 7.39-7.51 (3 H, m), 7.62-7.66 (2 H, m), 7.72 (2 H, d,  $J$  10.5 and 2.1), 8.05 (2 H, d,  $J$  8.3), 8.17 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 31.2, 36.3, 41.4, 84.0, 104.3, 116.1, 127.3, 127.8, 128.5, 129.1, 130.6, 131.6, 139.5, 145.6, 153.5, 161.0,

**(E)-Butyl 2-cyano-3-(3,4-dichlorophenyl)acryloylcarbamate (36).** Yield: 32%; white needles; Mp 157-58 °C (from ethanol); (Found: C, 52.75; H, 4.20; N, 8.27. Calc. for  $\text{C}_{15}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}_3$ : C, 52.80; H, 4.14; N, 8.21%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 0.93-0.97 (3 H, m), 1.39-1.44 (2 H, m), 1.65-1.72 (2 H, m), 4.23-4.27 (2 H, m), 7.59 (1 H, d,  $J$  8.6), 7.84 (1 H, dd,  $J$  2.2 and 8.6), 8.01 (1 H, d,  $J$  2.2), 8.23 (1 H, br s), 8.28 (1 H, s);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 13.7, 19.0, 30.6, 67.0, 105.0, 115.6, 123.6, 131.1, 131.6, 132.8, 134.2, 138.3, 150.1, 153.1, 157.5.

**(E)-Ethyl 2-cyano-3-(3,4-dichlorophenyl)acryloyl(methyl)carbamate (37).** Yield: 31%; white crystals; Mp 125-126 °C (from ethanol); (Found: C, 51.47; H, 3.58; N, 8.48. Calc. for  $\text{C}_{14}\text{H}_{12}\text{Cl}_2\text{N}_2\text{O}_3$ : C, 51.40; H, 3.70; N, 8.56%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.36 (3 H, t,  $J$  7.1), 3.30 (3 H, s), 4.36 (2 H, q,  $J$  7.1), 7.55 (1 H, d,  $J$  8.4), 7.69 (1 H, s), 7.80 (1 H, dd,  $J$  8.5 and 2.2), 7.94 (1 H, d,  $J$  2.1);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 14.4, 33.0, 64.3, 111.0, 115.0, 128.9, 131.2, 131.8, 132.0, 133.7, 136.7, 147.9, 153.9, 165.7.

**(E)-Ethyl 2-cyano-3-(3,4-dichlorophenyl)acryloyl(ethyl)carbamate (38).** Yield: 21%; pale yellow crystals; Mp 77-78 °C (from ethanol); (Found: C, 52.80; H, 3.99; N, 8.09. Calc. for  $\text{C}_{15}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}_3$ : C, 52.80; H, 4.14; N, 8.21%);  $\delta_{\text{H}}$  (300 MHz,  $\text{CDCl}_3$ ) 1.27 (3 H, t,  $J$  7.0), 1.37 (3 H, t,  $J$  7.1), 3.84 (2 H, q,  $J$  7.0), 4.37 (2 H, q,  $J$  7.1), 7.56 (1 H, d,  $J$  8.4), 7.70 (1 H, s), 7.81 (1 H, dd,  $J$  8.4 and 2.2), 7.94 (1 H, d,  $J$  2.1);  $\delta_{\text{C}}$  (75 MHz,  $\text{CDCl}_3$ ) 14.1, 14.4, 41.8, 64.2, 111.2, 115.0, 128.9, 131.2, 131.8, 132.0, 133.7, 136.7, 147.9, 153.7, 165.4.

**(E)-Ethyl butyl(2-cyano-3-(3,4-dichlorophenyl)acryloyl)carbamate (39).** Purified by flash chromatography (heptane/ethyl acetate, 80:20) followed by recrystallization from toluene. Yield: 18%; white crystals; Mp 39-40 °C (from toluene); (Found: C, 54.94; H, 4.81; N, 7.60. Calc. for C<sub>17</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C, 55.30; H, 4.91; N, 7.59%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>); 0.97 (3 H, t, *J* 7.3), 1.32-1.44 (5 H, m), 1.58-1.68 (2 H, m), 3.78 (2 H, m), 4.36 (2 H, q, *J* 7.1), 7.55 (1 H, d, *J* 8.4), 7.71 (1 H, s), 7.81 (1 H, dd, *J* 8.5 and 2.2), 7.94 (1 H, d, *J* 2.1);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.0, 14.4, 20.2, 30.9, 46.3, 64.2, 111.2, 115.0, 128.9, 131.3, 131.8, 132.1, 133.7, 136.7, 148.0, 154.0, 165.5,

**(E)-Ethyl benzyl(2-cyano-3-(3,4-dichlorophenyl)acryloyl)carbamate (40).** Yield: 55%; white solid; Mp 94-95 °C (from toluene); (Found: C, 59.45; H, 3.88; N, 6.90. Calc. for C<sub>20</sub>H<sub>16</sub>Cl<sub>2</sub>N<sub>2</sub>O<sub>3</sub>: C, 59.57; H, 4.00; N, 6.95%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.30 (3 H, t, *J* 7.1), 4.34 (2 H, q, *J* 7.1), 4.96 (2 H, s), 7.29-7.36 (5 H, m), 7.76 (1 H, s), 7.56 (1 H, d, *J* 8.4), 7.81 (1 H, dd, *J* 8.5 and 2.2), 7.94 (1 H, d, *J* 2.2);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.2, 49.6, 64.5, 110.8, 115.0, 127.9, 127.9, 128.7, 128.9, 131.3, 131.7, 132.1, 133.7, 136.4, 136.9, 148.6, 153.8, 165.5.

**(E)-ethyl 2-cyano-3-phenylacryloylcarbamate (41).** Yield: 84%; light-yellow crystals; Mp 136-37 °C (from ethyl acetate); (Found: C, 63.94; H, 4.90; N, 11.55. Calc. for C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>: C, 63.93; H, 4.95; N, 11.47%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.36 (3 H, t, *J* 7.1), 3.34 (2 H, q, *J* 7.1), 7.51-7.55 (2 H, m), 7.58-7.62 (1 H, m), 7.97-7.99 (2 H, m), 8.26 (1 H, br s), 8.42 (1 H, s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 63.3, 102.9, 116.2, 129.4, 131.2, 131.2, 133.9, 150.0, 156.2, 158.0.

**(E)-Ethyl 3-(4-chlorophenyl)-2-cyanoacryloylcarbamate (42).** Yield: 66%; white crystals; Mp 189-191 °C (from ethanol); (Found: C, 56.13; H, 3.79; N, 10.02. Calc. for C<sub>13</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>3</sub>: C, 56.03; H, 3.98; N, 10.05%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.12 (3 H, t, *J* 7.0), 4.04 (2 H, q, *J* 7.2), 7.23 (2 H, d, *J* 8.5), 7.67 (2 H, d, *J* 8.7), 7.91 (1 H, s), 10.00 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.5, 61.2, 105.8, 114.4, 128.5, 129.1, 131.0, 137.8, 150.3, 150.4, 160.0.

**(E)-Ethyl 2-cyano-3-(3,4-dimethylphenyl)acryloylcarbamate (43).** Yield: 24%; pale yellow crystals; Mp 131-132 °C (from ethanol); (Found: C, 66.11; H, 5.81; N, 10.22. Calc. for C<sub>15</sub>H<sub>16</sub>N<sub>2</sub>O<sub>3</sub>: C, 66.16; H, 5.92; N, 10.29%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.35 (3 H, t, *J* 7.1), 2.34 (6 H, d, *J* 7.0), 4.30 (2 H, q, *J* 7.1), 7.26 (1 H, d, *J* 7.6), 7.71-7.76 (2 H, m), 8.19 (1 H, br s), 8.33 (1 H, s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 20.0, 20.6, 63.0, 101.0, 116.7, 129.1, 130.7, 132.6, 138.0, 144.4, 150.0, 156.4, 158.2.

**(E)-Ethyl 2-cyano-3-(4-methoxyphenyl)acryloylcarbamate (44).** Yield: 73%; yellow crystals; Mp 164-165 °C (from ethanol); (Found: C, 61.35; H, 5.09; N, 10.22. Calc. for C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>: C, 61.31; H, 5.14; N, 10.21%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 8.33 (1 H, s), 8.18 (1 H, br s), 7.99 (2 H, d, *J* 8.9), 7.00 (2 H, d, *J* 8.9), 4.31 (2 H, q, *J* 7.1), 3.91 (3 H, s), 1.36 (3 H, t, *J* 7.1);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.4, 55.9, 62.9, 98.9, 115.0, 117.1, 124.2, 134.0, 150.1, 155.6, 158.4, 164.3.

**(E)-Ethyl 2-cyano-3-(3-fluoro-4-(trifluoromethyl)phenyl)acryloylcarbamate (45).** The reaction was stirred at room temperature for 1 hour. Yield: 18%; white crystals; Mp 196-197 °C (from toluene); (Found: C, 50.68; H, 2.87; N, 8.35. Calc. for C<sub>14</sub>H<sub>10</sub>F<sub>4</sub>N<sub>2</sub>O<sub>3</sub>: C, 50.92; H, 3.05; N, 8.48%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.32 (3 H, t, *J* 7.1), 4.23 (2 H, q, *J* 7.1), 7.76-7.89 (3 H, m), 8.20 (1 H, s), 11.07 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.1, 60.6, 109.2, 113.2, 116.3, 116.6, 125.0, 126.6, 136.2, 136.3, 147.6, 149.7, 156.0, 159.3.

**(E)-Ethyl 3-(3-bromo-4-nitrophenyl)-2-cyanoacryloylcarbamate (46).** Yield: 34%; white crystals; Mp 192-193 °C (from toluene); (Found: C, 42.41; H, 2.74; N, 11.41. Calc. for C<sub>13</sub>H<sub>10</sub>BrN<sub>3</sub>O<sub>5</sub>: C, 42.41; H, 2.74; N, 11.23%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 0.96 (3 H, t, *J* 7.1), 3.88 (2 H, q, *J* 7.1), 7.59 (1 H, d, *J* 8.4), 7.68 (1 H, dd, *J* 8.5 and 1.8), 7.76 (1 H, s), 7.85 (1 H, d, *J* 1.7), 10.51 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 13.3, 61.0, 110.0, 113.2, 113.3, 124.9, 128.5, 135.1, 135.4, 147.0, 149.6, 150.0, 159.3.

**(E)-Ethyl 3-(4-chloro-3-nitrophenyl)-2-cyanoacryloylcarbamate (47).** Yield: 12%; yellow crystals; Mp 177-178 °C (from ethanol); (Found: C, 48.16; H, 2.92; N, 12.92. Calc. for C<sub>13</sub>H<sub>10</sub>ClN<sub>3</sub>O<sub>5</sub>: C, 48.24; H, 3.11; N, 12.98%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.22 (3 H, t, *J* 7.1), 4.15 (2 H, q, *J* 7.1), 7.57 (1 H, d, *J* 8.5), 8.02 (1 H, dd, *J* 8.5 and 2.2), 8.05 (1 H, s), 8.25 (1 H, d, *J* 2.2), 10.17 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.2, 62.4, 109.0, 114.4, 127.2, 130.5, 131.1, 132.1, 132.6, 133.5, 148.8, 150.8, 159.4.

**(E)-Ethyl 3-(4-bromo-3-nitrophenyl)-2-cyanoacryloylcarbamate (48).** Yield: 34%; pale orange crystals; Mp 179-181 °C (from ethanol); (Found: C, 42.68; H, 2.54; N, 11.42. Calc. for C<sub>13</sub>H<sub>10</sub>BrN<sub>3</sub>O<sub>5</sub>: C, 42.41; H, 2.74; N, 11.41%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.21 (3 H, t, *J* 7.1), 4.14 (2 H, q, *J* 7.1), 7.75 (1 H, d, *J* 8.4), 7.92 (1 H, dd, *J* 8.5 and 2.1), 8.02 (1 H, s), 8.19 (1 H, d, *J* 2.1), 10.20 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.2, 62.4, 109.1, 114.4, 127.1, 131.7, 132.6, 133.3, 135.3, 135.8, 148.9, 150.8, 159.4.

**(E)-Ethyl 3-(4-chloro-3-(trifluoromethyl)phenyl)-2-cyanoacryloylcarbamate (49).** Yield: 50%; white crystals; Mp 164-166 °C (from ethanol); (Found: C, 48.58; H, 2.69; N, 8.04. Calc. for C<sub>14</sub>H<sub>10</sub>ClF<sub>3</sub>N<sub>2</sub>O<sub>3</sub>: C, 48.50; H, 2.91; N, 8.08%);  $\delta_{\text{H}}$  (300 MHz, CDCl<sub>3</sub>) 1.28 (3 H, t, *J* 7.1), 4.23 (2 H, q, *J* 7.1), 7.58 (1 H, d, *J* 8.3), 8.06-8.11 (2 H, m), 8.14 (1 H, s), 9.63 (1 H, br s);  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 14.1, 62.2, 107.8, 114.6, 120.0, 123.6, 129.6 (q, *J* 5.3), 130.1, 132.2, 133.5, 136.1, 149.9, 150.7, 159.6.

## DENSITY FUNCTIONAL THEORY (DFT) CALCULATIONS

The exchange functional by Becke combined with the exchange functional of Lee, Yang and Parr (B3LYP)<sup>6</sup> was chosen since this has been tested and found reliable in a series of related systems.<sup>7</sup> <sup>8</sup> Basis sets of the correlation consistent type was used (cc-pVXZ, X=[D,T,Q]).<sup>9</sup> These basis sets have been tested on similar systems with good results,<sup>10</sup> and allows for extrapolation towards the basis set limit.<sup>11</sup> The cc-pVQZ basis set has been used to test the quality of this extrapolation procedure. All calculations were performed with the Gaussian-03 package<sup>12</sup> using standard convergence criteria. No constraints were enforced during the optimizations. Solvent effects have not been included. For all molecules 3-6 different starting configurations were relaxed using cc-pVDZ basis set to ensure that the potential energy surface surrounding the expected minimum had been well examined. The converged cc-pVDZ structures were taken as starting points for cc-pVTZ. The increased basis set did not give rise to any noticeable change in the geometry. The sign of the calculated energy difference is evident and consistent already at the cc-pVDZ level, but in order to reduce the basis set error to within 1 kJ/mol the cc-pVTZ basis set was included (e.g. the compound **12** test case, where a single point cc-pVQZ calculation on the cc-pVTZ optimized structure, serves as reference). The exchange-correlation functionals of Perdew and Wang (PW91)<sup>13</sup> were used to check for consistency between these two widely trusted functionals with the cc-pVTZ basis set, also for **12**. The difference in the PW91 and B3LYP functionals is negligible, and we conclude that either of these would be equally reliable for calculations on these systems. All DFT results are summarized in Table S1.

## X-RAY CRYSTALLOGRAPHIC ANALYSIS

Faintly yellowish single crystals of **41** were crystallized from ethanol. Crystal data: C<sub>13</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>, *M<sub>r</sub>* = 244.25, monoclinic, space group *P*2<sub>1</sub>/*n* (No. 14), *a* = 4.7990(11) Å, *b* = 13.610(3) Å, *c* = 18.352(4) Å,  $\beta$  = 89.969(18), *V* = 1198.6(5) Å<sup>3</sup>, *Z* = 4, *D<sub>c</sub>* = 1.353 Mg m<sup>-3</sup>, *F*(000) = 512,  $\mu(\text{Mo K}\alpha)$  = 0.098 mm<sup>-1</sup>, *T* = 122.0 (5) K, crystal dimensions = 0.38 x 0.11 x 0.06 mm. Diffraction data were

collected on an Enraf-Nonius KappaCCD diffractometer using graphite monochromated Mo  $K\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ).<sup>14</sup> The reflections were measured in the range  $-6 \leq h \leq 4$ ,  $-17 \leq k \leq 17$ ,  $-23 \leq l \leq 23$ , ( $5.01^\circ < \theta < 27.51^\circ$ ). Data were reduced using the program EvalCCD.<sup>15, 16</sup> A total of 20257 reflections were averaged according to the point group symmetry 2/m resulting in 2747 unique reflections ( $R_{\text{int}} = 0.0447$  on  $F_o^2$ ). Absorption correction was applied using the program NUMABS ( $T_{\text{min}} = 0.972$ ;  $T_{\text{max}} = 0.995$ ) as part of the program maXus.<sup>17, 18</sup> The structure was solved by the direct method in the programme SHELXS97<sup>19, 20</sup> and refined using the programme SHELXL97.<sup>21</sup> Full matrix least-squares refinement on  $F^2$  was performed, minimizing  $\sum w(F_o^2 - F_c^2)^2$ , with anisotropic displacement parameters for the non-hydrogen atoms. Refinement of the structure turned out to be problematic. The unit-cell parameters reveal that  $\beta$  is very close to  $90^\circ$ . Pseudomerohedral twinning was included with the twinning operator  $(-1 \ 0 \ 0, 0 \ -1 \ 0, 0 \ 0 \ 1)$ , and the structure could now be satisfactorily refined with a twinning fraction of 0.46, indicating a nearly perfect twinned crystal. The positions of all hydrogen atoms were located on intermediate difference electron density maps. The hydrogen atoms connected to the nitrogen atom and to the double bond were refined with fixed isotropic displacement parameters, while the rest of the hydrogen atoms were included in calculated positions, riding on the parent atoms with fixed isotropic displacement parameters. The refinement (171 parameters, 2747 reflections) converged at  $R_F = 0.0303$ ,  $wR_{F2} = 0.0632$  for 2465 reflections with  $F_o > 4\sigma(F_o)$ ;  $w = 1/[\sigma^2(F_o^2) + (0.0293P)^2 + 0.2261P]$ , where  $P = (F_o^2 + 2F_c^2)/3$ ;  $S = 1.027$ . In the final difference Fourier map maximum and minimum electron densities were 0.188 and  $-0.155 \text{ e \AA}^{-3}$ , respectively. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.<sup>21, 22</sup> Fractional atomic coordinates, list of anisotropic displacement parameters and a complete list of geometrical data have been deposited in Cambridge Crystallographic Data Centre (No. CCDCxxxxxx).\*

## FLUORESCENCE POLARIZATION ASSAY

Competition fluorescence polarization assays were performed as previously described.<sup>23-25</sup> Briefly for the PICK1 PDZ domain,  $K_i$  values were determined using a fixed concentration of fluorescently (Oregon Green) labelled peptide corresponding to the 13 C-terminal residues of the hDAT peptide (40 nM), a fixed non-saturating concentration of purified PICK1 (0.45  $\mu\text{M}$ ) and

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\* CCDC xxxxxx contains the supplementary crystallographic data. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

increasing concentrations of compound to be tested. Equilibrium competition binding isotherms were constructed by plotting fluorescence polarization versus the concentration of compound.<sup>23, 24</sup> Competition fluorescence polarization assays for PDZ1, PDZ2, and PDZ3 from PSD-95 were performed using a similar procedure as that described for PICK1.<sup>25</sup> To detect binding to PDZ1 and PDZ2, a peptide corresponding to the last 11 residues of the NMDA receptor subunit (NR2B) was synthesized through a CSG tripeptide linker and labelled at the *N*-terminus with Cy5-maleimide (GE Healthcare, UK). To detect binding to PDZ3, a peptide corresponding to the to the last 11 residues of CRIPT was synthesized and labelled at the *N*-terminus with Cy5. All peptides have been synthesized as previously described.<sup>25</sup>

### **PULL-DOWN ASSAY**

A fusion between GST and the 24 C-terminal amino acids of hDAT (GST DAT C24) was expressed in BL21(DE3)pLysS (Novagen) and purified on glutathione-coated sepharose beads (Pharmacia) as previously described.<sup>24</sup> For the pull-down, 5  $\mu$ L dry volume of beads with bound GST or GST DAT C24 were suspended in 200  $\mu$ L TBS buffer containing 0.5  $\mu$ M purified PICK1. Compounds to be tested were added in DMSO to a final concentration of 50-200  $\mu$ M (Final DMSO concentration was 2%) and incubated. Bound protein was eluted in loading buffer and analyzed by 12% SDS-PAGE and stained with Gelcode Blue stain reagent (Pierce). The degree of PICK1 pull-down was quantified by densitometry analysis of the stained gels.<sup>24</sup>

### **MOLECULAR MODELLING AND DOCKING**

Compounds were docked into the human PICK1 PDZ domain (PDB ID: 2GZV) using previously described methods.<sup>24</sup> Briefly, Autodock 4.01 was used to perform flexible and rigid docking calculations. All waters were removed and an energy grid for the docking approach was generated with the program AutoGrid with dimensions of 60  $\text{\AA}$   $\times$  60  $\text{\AA}$   $\times$  60  $\text{\AA}$  and 0.375  $\text{\AA}$  grid spacing. AutoDock 4 was used to evaluate relative ligand binding energies over the conformational search space using the Lamarckian genetic algorithm-local search (GALS) method, and a low-frequency local search method was applied to docking solutions to find the local minimum. 100 conformations were obtained from docking for each compound, and these were clustered and subjected to visual inspection for appropriate chemical geometry. Pymol, version 0.97, was used to generate figures.<sup>26</sup>



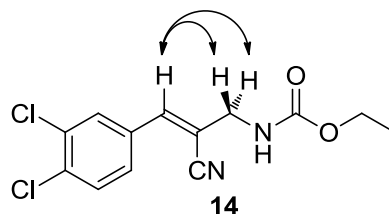
## ANALYSIS OF X-RAY, NMR AND DFT CALCULATIONS FOR *E/Z* DETERMINATIONS

### Configuration of **1** and **41**

The structure determination and subsequent refinement of **41** unequivocally demonstrated that compound **41** possesses the *E*-configuration around the double bond (Fig. 3). In order to exclude discrepancies from the difference in the aromatic moiety between **1** and **41**, we performed quantum mechanical modelling using density functional theory (DFT). The X-ray structure and the optimized structure from DFT calculations of (*E*)-**41** differed only slightly. The structure from DFT calculation was entirely planar, whereas the ethyl ester of the X-ray determined structure is bended, such that the C12-C13 bond is at an angle of approximately 60° to the plane spanned by the aromatic group (Fig. 3). This bending is a result of crystal packing interactions, and the planar conformation is expected to be the predominant species in solution. Subsequently, the (*Z*)-**41** regioisomer was analyzed by DFT calculations, and it was found that this configuration was destabilized by ca. 25 kJ/mol, compared to the *E*-configuration, which is in accordance with the results of the X-ray structure determination. Then, in order to determine whether the *E*- or *Z*-isomer is formed during the Knoevenagel reaction in the synthesis of **1**, we performed DFT calculations similar to those performed with **41**. We found, that by far the most stable configuration of **1** is the *E*-isomer, as (*E*)-**1** is stabilized by ca. 23 kJ/mol compared to the (*Z*)-**1** isomer. We thereby conclude that the chlorine atoms on the aromatic ring do not influence the stabilization of the double bond and that the *E*-configuration is formed exclusively via the Knoevenagel reaction for **1**.

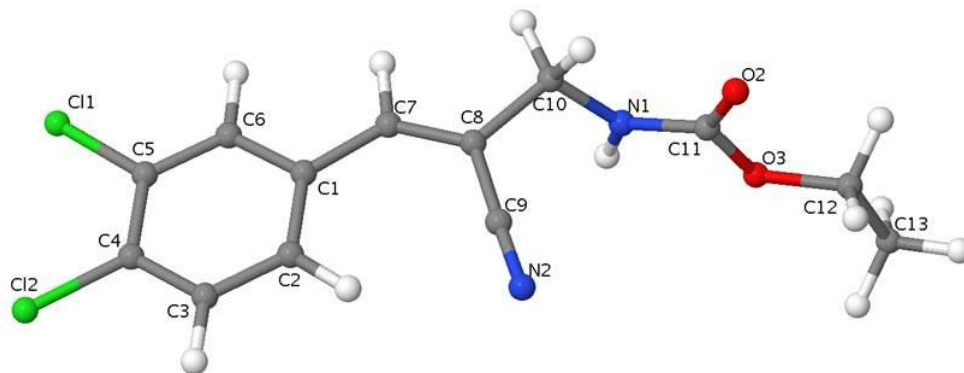
### Configuration of **10**, **12** and **14**

The *Z* geometry of the double bond in **14** was verified by NOESY experiments, where a dipolar coupling between the vinylic proton and the protons of the methylene group was observed (Fig. S1).



**Fig. S1** The *Z*-configuration of the double bond in **14** was verified by NOESY experiments, where a dipolar coupling between the vinylic proton and the protons of the methylene group was observed.

Further, the regiochemistry of **14**, was studied via DFT calculations and the results revealed that (*Z*)-**14** was stabilized by ca. 8 kJ/mol compared to (*E*)-**14** (Fig. S2), in agreement with the geometry observed by NMR.



**Fig. S2** DFT optimized structure of (*Z*)-**14** at the B3LYP/cc-pVTZ level. This configuration is stabilized by 8 kJ/mol compared to the *E*-configuration, in accordance with NOESY experiments.

For the precursors of **10** and **12** the formed isomers also possessed geometry with the aromatic and the cyano group on the same side of the double bond (*Z*) according to DFT calculations and the energy difference relative to the other isomer was found to be ca. 7 and ca. 10 kJ/mol for compounds **10** and **12**, respectively (Table S1). These energy differences are not sufficiently large to account for the observed (>99%, NMR) *E/Z* specificity of these compounds, but are still in accordance with the experimental findings.

**Table S1**

Compound	<b>1</b>	<b>41</b>	<b>14</b>	<b>10</b>	<b>12</b>
B3LYP/cc-pVDZ	19.68	21.38	9.68	5.98	7.54
B3LYP/cc-pVTZ	22.24	23.95	8.49	6.94	9.55
PW91/cc-pVTZ	-	-	-	-	9.56
Extrapolation	23.32	25.03	7.99	7.34	10.40

<sup>a</sup> Single point calculation

**Table S1.** Energy of the conformation with the aromatic and cyano groups on opposite sides relative to the conformation with the aromatic and cyano groups on the same side of the double bond in kJ/mol. In all cases, the conformation with the aromatic and cyano group on the same side of the double bond is more stable. See Fig. 3 and S2. The extrapolation is performed from cc-pVDZ and cc-pVTZ basis sets with B3LYP.

In summary, experimental evidence and calculations for compounds **1**, **41**, **14**, **10** and **12** verify that only one isomer is present, and that this is the isomer with the cyano and the aromatic unit on the same side of the double bond regardless of the synthetic method, Knoevenagel or aminolysis, or of the third substituent on the double bond. This suggests that all derivatives of **1** described in this paper will be found exclusively in this conformation as illustrated.

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