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The use of microwave radiations and propylphosphonic anhydride (T3P®) for rapid and highly yielding amide bond formation and peptide coupling.

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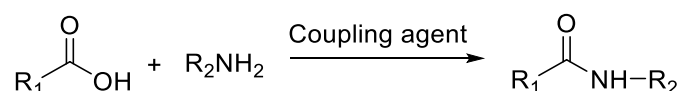
Abstract

An improved, fast, and efficient procedure for amide bond formation and peptide coupling is reported leading to the expected corresponding derivatives in good to excellent isolated yields ranging from 26 to 90%. The outcome of the reaction was also investigated in relation to the influence of different parameters such as the nature of the solvent, the temperature as well as the amine/carboxylic acid couple involved or the hydrolysis conditions.

Keywords: Amide bond formation – Peptide coupling – Microwave reaction – propylphosphonic anhydride.

Introduction

The amide bond formation is one of the most fundamental and important reactions encountered in synthetic organic chemistry¹⁻³ (Scheme 1).



Scheme 1. General scheme of amide bond formation

Different methods are described in literature and amides are usually prepared by coupling of carboxylic acids and amines using either a coupling reagent or by prior conversion of the carboxylic acid into a derivative. Nevertheless, these methods suffer from considerable drawbacks such as high cost, toxicity, and production of large amounts of waste.⁴⁻⁶

Furthermore, in the pharmaceutical industry, construction of the amide group constitutes one of the most pivotal and frequently performed reactions. Thus, investigation on general methods for catalytic/sustainable amide or peptide formation has been defined in 2018 as a priority in the ten Key Green Chemistry Research Areas by the ACS Green Chemistry Institute® Pharmaceutical Roundtable (GCIPR).⁷

In this context an ideal coupling agent should form the expected amide bond formation under mild reaction conditions and minimize by-product formation.

T3P® is a registered trademark for *n*-propylphosphonic anhydride (Figure 1) and has been explored as a mild and chemoselective peptide coupling reagent. Nevertheless, the amide bond formation with T3P can take several hours at room temperature. During the last decade, Microwave Assisted Organic Synthesis (MAOS) has become a widely useful tool to help with reaction optimization including better selectivity, reduction in reaction time and improving yields.⁸⁻¹²

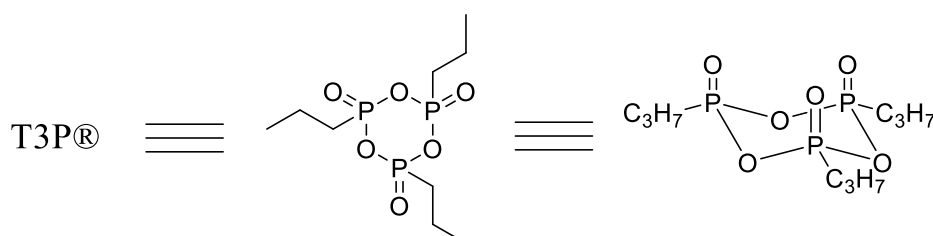


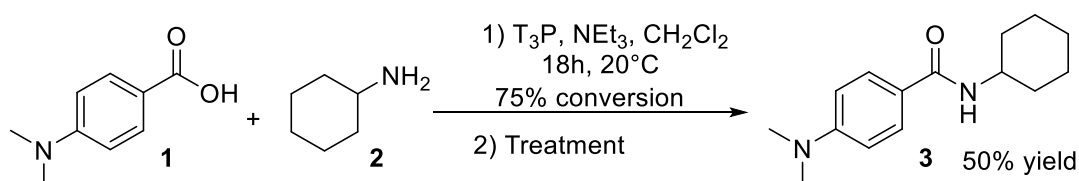
Figure 1. Structure of *n*-propylphosphonic anhydride (T3P®)

Herein, we will report an improved and fast microwave procedure for amide bond formation and peptide coupling involving T3P as coupling agent as well as study the influence of the experimental conditions on the outcome of the reaction.

Results and discussion

Stable and affordable the *n*-propylphosphonic anhydride (T3P) is often sold as a 50% (w/w) solution in a wide range of different solvents (DMF, DCM, THF or ethylacetate).^{13,14} It is currently used extensively in solution or liquid phase synthesis for amide bond formation, including on large scales.¹⁵⁻¹⁷ Despite the efficient coupling capacity of T3P® and low racemization rates¹⁸, to the best of our knowledge, no rapid microwave conditions for such a reaction using this coupling reagent have been reported to date. Thus, we have developed a straightforward microwave protocol for amidation reactions.

Recently, we have been able to use T3P for amide bond formation between dimethyl aminobenzoic acid **1** and cyclohexylamine **2** but with moderate conversion (75%, 18 h, 20°C) and moderate isolated yield (50%) (Scheme 2).



Scheme 2. Room temperature T3P amide bond formation between dimethyl aminobenzoic acid **1** and cyclohexylamine **2**

We have encountered challenges in enhancing these outcomes and elevating the conversion rate, hindering us from envisioning a robust response for a large-scale synthesis using costly starting materials. Given these discouraging results, we have explored the feasibility of conducting the reaction under microwave irradiation conditions. Our first attempt investigated coupling reaction between dimethyl aminobenzoic acid **1** and cyclohexylamine **2** under different conditions using microwave irradiation (450W) as illustrated in Figure 2.

Thus, the best conditions consist in performing the reaction in dichloromethane (figure 2A) for 20 minutes at 120°C (Figure 2B) and by extracting the crude reaction mixture by a 10% NaHCO₃ solution (Figure 2C). Under these experimental conditions, the expected compound **3** was obtained in 93% isolated yield (Figure 2).

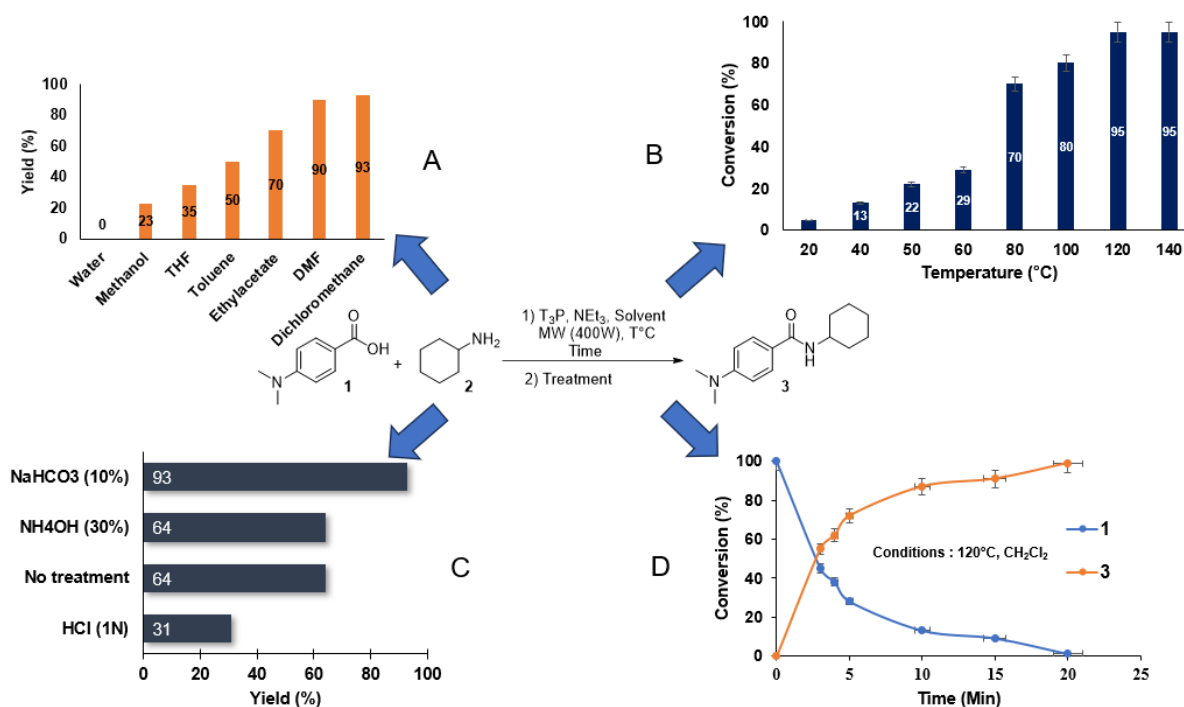


Figure 2. Evaluation of the influence of different parameters on the outcome of the T3P amide bond formation between dimethyl aminobenzoic acid **1** and cyclohexylamine **2**. (A) Influence of the nature of the solvent and (B) of the temperature on the conversion of the reaction. (C) Influence of the work-up carried out after the reaction. (D) Kinetic study of T3P amide bond formation between dimethyl aminobenzoic acid **1** and cyclohexylamine **2** under microwave conditions.

More precisely, a kinetic study of this reaction indicates that a conversion of around 100% is obtained after 20 minutes demonstrating the efficiency of this methodology compared to classic heating conditions (Figure 2D). Reasons for such an improvement could come from the fact that microwave heating and conduction heating have different underlying principles and thermal management considerations. Microwave heating relies on

electromagnetic waves and can provide rapid and volumetric homogenic heating, while conduction heating involves direct physical contact for heat transfer between two areas with different temperatures leading to a non-homogenic temperature of reaction.¹⁹⁻²³

It also clearly appears that the nature of the solvent used has a dramatic effect on the conversion varying from 0 to 90% (Figure 2A). Thus, solvents such as DMF or dichloromethane led both to a conversion of 90%, whereas low conversions were observed by performing the reaction in methanol, THF or toluene. On the other hand, the influence of the temperature is important since better results are obtained at 120°C leading in this case to a 93% optimal conversion (Figure 2B). Furthermore, the impact of the quenching experimental conditions was assessed, leading to an enhanced isolated yield achieved through hydrolysis and extraction using a 10% NaHCO₃ solution. Moreover, this reaction does not require any flash chromatography purification step as demonstrated by recording ¹³C NMR spectra of the crude reaction mixture before and after hydrolysis/extraction step with a 10% NaHCO₃ solution followed by drying over Na₂SO₄ (Figure 3).

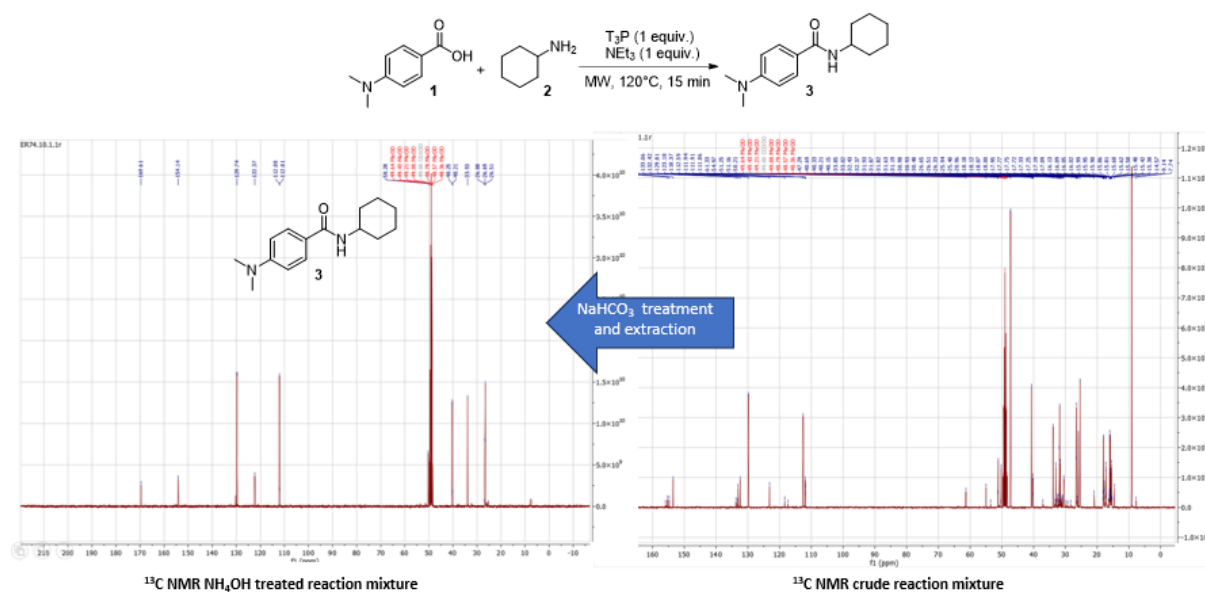
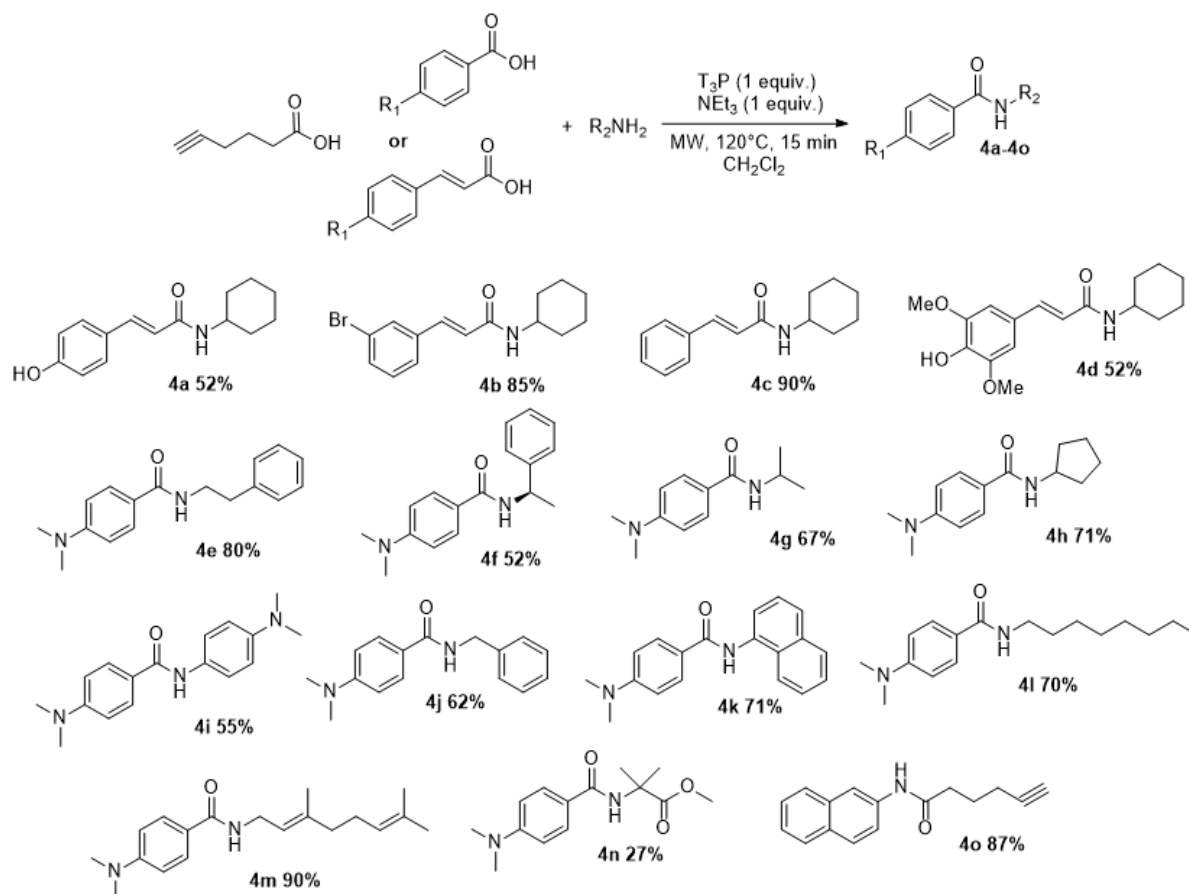


Figure 3. ¹³C NMR spectra of the T3P amide bond formation crude reaction mixture between dimethyl aminobenzoic acid **1** and cyclohexylamine **2** before and after hydrolysis / extraction with a 10% NaHCO₃ solution

Additionally, performing the reaction with various carboxylic acids or amine afforded in all cases the expected amide compounds in good to excellent chemical yields varying from 52 to 90% (Scheme 3).



Scheme 3. Examples and yields of amide compounds synthesized by using microwave radiations and T3P®.

On the other hand, we have investigated the ability of such a coupling methodology between an amine and a bile acid. In this case, the amide derivative **6** was obtained in 90% yield without any purification after the hydrolysis/extraction step. As evidence, the ¹³C NMR spectra of the crude reaction mixture before and after hydrolysis/extraction were recorded (Figure 4).

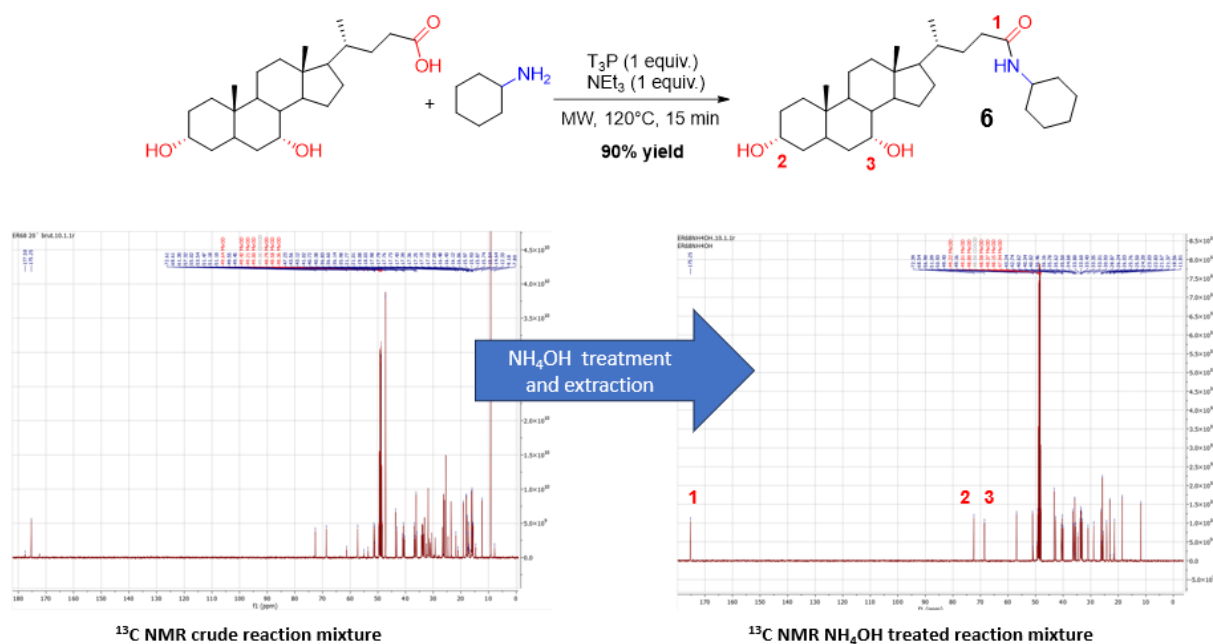


Figure 4. ¹³C NMR spectra of the T₃P amide bond formation crude reaction mixture between chenodeoxycholic acid and cyclohexylamine before and after hydrolysis / extraction with a NH₄OH solution (30% v/v).

Finally, the scope and limitation of this methodology was investigated in the coupling reaction between protected amino acids as illustrated on the chosen examples outlined in Figure 5. Thus, the expected peptides **7a-7d** were obtained in non-optimized chemical yields ranging from 28 to 70%. Notably, the formation of **7a** and **7d** exclusively occurred in a single diastereomeric form, as confirmed through ¹H and ¹³C NMR analyses, as well as HPLC analysis (refer to the supporting information). As anticipated, only two diastereomers were observed for **7b**, aligning with our use of a 4-hydroxy-L-proline precursor (60/40). In all three examples, racemization was not encountered. Similarly, for derivative **7c**, only one enantiomer was observed, with no traces of racemization detected (see supporting information).

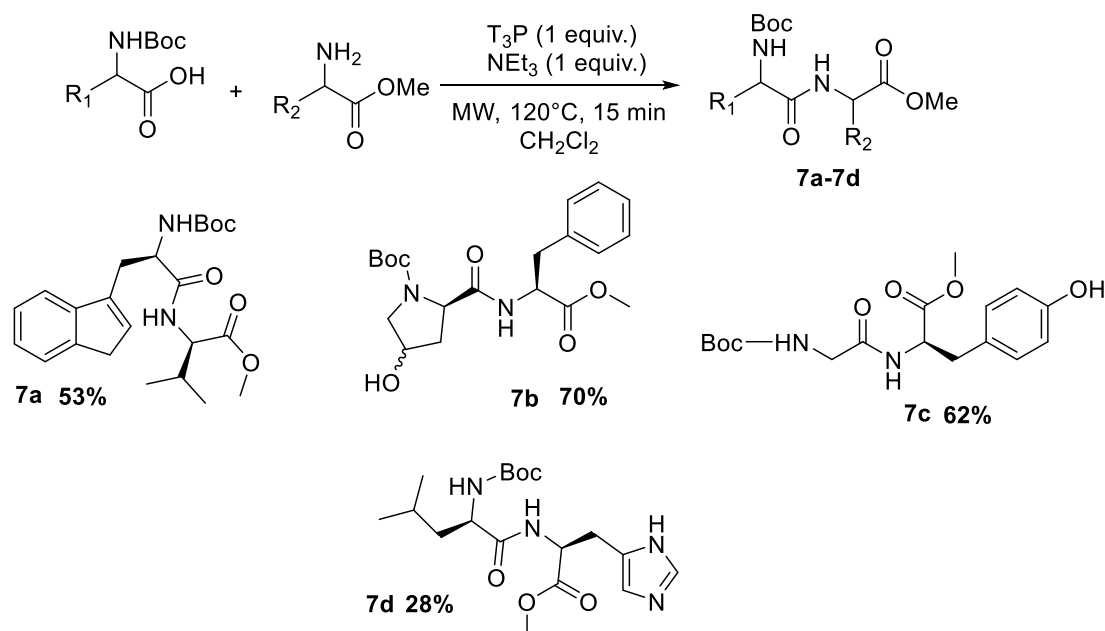


Figure 5. Examples of use of microwave radiations and T3P® for peptide coupling reaction.

Conclusion

An easy and efficient amide bond formation and peptide coupling system has been developed involving microwave radiations and the propylphosphonic anhydride (T3P®) reagent. Studies are now under current investigation for application of such methodology on more complex molecules. Results will be reported in due course.

Experimental section

All solvents were purified according to reported procedures, and reagents were used as commercially available. Methanol, ethyl acetate, dichloromethane, ammonia, and petroleum ether (35–60 8C) were purchased from Sigma-Aldrich and used without further purification. Column chromatography was performed on Macherey-Nagel silica gel (70–230 mesh). ^1H and ^{13}C NMR spectra were recorded on Bruker AC400 spectrometers working at 400 MHz and 100 MHz, respectively (the usual abbreviations are used: s: singlet, d: doublet, t: triplet, q: quadruplet, m: multiplet). The chemical shifts (ppm) were determined relative to Me_4Si (^1H and ^{13}C). The purity of the compounds was checked by analytical Agilent 1100 series HPLC (C18 column, eluent CH_3CN -water-TFA (90:10:0.025, v/v/v), 0.5-1 mL/min) with PDA detector spanning from 210 nm to 310 nm. All compounds described possessed purity above 95%.

Kinetic study of N-cyclohexyl-4-(dimethylamino)benzamide 3 synthesis

In a 5-10 mL microwave reaction vial were introduced in 5 mL of dichloromethane, 82 mg of dimethylamino benzoic acid **1** (0.5 mmol), 70 mg of cyclohexylamine **2** (0.7 mmol), 70 mg of NEt₃ (0.7 mmol) and 318 mg (297 μ L) of T3P (50 wt.% in ethylacetate) (0.7 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (450 Watt) at 120 °C, during various times (2, 3, 4, 5, 10, 15, 20 minutes) using a normal mode and a pre-stirring of 20 seconds. The reaction was then quenched by adding 1 mL of a 10% NaHCO₃ solution. The conversion was estimated by HPLC analysis of the organic phase performed on an Agilent 1100 series apparatus (C18 column, eluent MeOH (TFA 0.1%)-Water (TFA 0.1%) with PDA detector spanning from 210 nm to 310 nm.

General procedure applied for the synthesis of derivatives **3**, **4a-4t**, **7a-7f**

Synthesis of N-cyclohexyl-4-(dimethylamino)benzamide **3**

In a 10-20 mL microwave reaction vial were introduced in 10 mL of dichloromethane, 165 mg of dimethylamino benzoic acid **1** (1 mmol), 140 mg of cyclohexylamine **2** (1.4 mmol), 140 mg of NEt₃ (1.4 mmol) and 636 mg (595 μ L) of T3P (50 wt.% in ethylacetate) (1.4 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (450 Watt) at 120 °C for 10 minutes using a normal mode and a pre-stirring of 20 seconds. The organic phase was then washed twice with 5 mL of 10% NaHCO₃ solution. The aqueous phases were extracted with 5 mL of dichloromethane and the combined organic phases were dried over Na₂SO₄, filtered, and concentrated *in vacuo* to afford the expected product N-cyclohexyl-4-(dimethylamino)benzamide **3** as a yellow oil in 59% yield (146 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.66 (m, 2H), 6.68 (m, 2H), 5.85 (m, 1H), 3.96 (dddd, $J = 3.93, 7.90, 10.58, 14.68$ Hz, 1H), 3.00 (s, 6H), 2.02 (dt, $J = 3.93, 12.50$ Hz, 2H), 1.69 (m, 2H), 1.42 (m, 2H), 1.21 (m, 3H). ¹³C NMR (101 MHz, MeOD) δ 169.61, 154.14, 129.74, 122.37, 112.08, 50.28, 40.25, 33.93, 26.69, 26.51. HRMS–ESI (m/z): calcd for C₁₅H₂₂N₂O ([M + H]⁺), 247.1766; found, 247.1736.

4a, Pale yellow oil, 52% yield (128 mg). ¹H NMR (400 MHz, CDCl₃) δ 6.93 (m, 1H), 6.70 (m, 1H), 6.23 (d, $J = 8.25$ Hz, 1H), 5.67 (d, $J = 15.60$ Hz, 1H), 5.47 (d, $J = 8.54$ Hz, 1H), 3.31 (m, 1H), 1.40 (td, $J = 3.72, 7.61, 8.27$ Hz, 2H), 1.16 (pd, $J = 3.97, 5.10, 7.59$ Hz, 3H), 1.05 (m, 2H), 0.79 (dddd, $J = 3.55, 9.56, 12.71, 15.94$ Hz, 2H), 0.62 (dddd, $J = 5.44, 10.45, 15.68, 19.67, 24.35$ Hz, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 166.26, 158.84, 141.24, 130.47,

129.59, 129.31, 129.00, 126.58, 117.75, 116.15, 48.72, 33.28, 25.63, 25.01. HRMS–ESI (m/z): calcd for C₁₅H₁₉NO₂ ([M + H]⁺), 246.1449; found, 246.1426.

4b, Yellow gum, 85% yield (262 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.63 (t, *J* = 1.85 Hz, 1H), 7.52 (d, *J* = 15.59 Hz, 1H), 7.41 (m, 2H), 7.21 (t, *J* = 7.86 Hz, 1H), 6.38 (d, *J* = 15.60 Hz, 1H), 5.70 (d, *J* = 8.20 Hz, 1H), 3.90 (dddd, *J* = 3.97, 8.03, 10.66, 14.68 Hz, 1H), 1.97 (m, 3H), 1.68 (ddt, *J* = 3.94, 12.85, 38.80 Hz, 4H), 1.39 (m, 2H), 1.19 (m, 4H). ¹³C NMR (101 MHz, CDCl₃) δ 164.40, 139.06, 137.14, 132.31, 130.32, 130.20, 126.60, 122.92, 122.67, 48.48, 33.20, 25.54, 24.86. HRMS–ESI (m/z): calcd for C₁₅H₁₈BrNO ([M + H]⁺), 309.0551; found, 309.0552.

4c, Pale yellow oil, 90% yield (207 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.56 (d, *J* = 15.63 Hz, 1H), 7.41 (dt, *J* = 3.50, 5.79 Hz, 2H), 7.27 (m, 3H), 6.39 (d, *J* = 15.61 Hz, 1H), 3.86 (tt, *J* = 4.01, 10.50 Hz, 1H), 1.93 (dt, *J* = 3.25, 10.55 Hz, 2H), 1.67 (dt, *J* = 3.85, 13.83 Hz, 2H), 1.58 (m, 1H), 1.23 (m, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 165.11, 140.61, 135.08, 129.55, 128.83, 127.80, 121.43, 121.38, 77.48, 77.16, 76.84, 48.49, 48.37, 33.29, 33.26, 25.63, 24.98. HRMS–ESI (m/z): calcd for C₁₅H₁₉NO ([M + H]⁺), 230.1500; found, 230.1503.

4d, Pale yellow oil, 52% yield (159 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.48 (d, *J* = 15.46 Hz, 1H), 6.70 (s, 2H), 6.27 (d, *J* = 15.45 Hz, 1H), 5.84 (s, 1H), 5.75 (d, *J* = 8.30 Hz, 1H), 3.84 (s, 6H), 1.96 (dt, *J* = 4.13, 12.49 Hz, 3H), 1.70 (dt, *J* = 3.81, 13.52 Hz, 2H), 1.60 (dt, *J* = 3.75, 12.99 Hz, 1H), 1.36 (m, 2H), 1.22 (m, 1H), 1.15 (m, 3H). ¹³C NMR (101 MHz, CDCl₃) δ 166.33, 147.26, 141.62, 136.53, 127.69, 119.25, 104.76, 56.89, 49.34, 33.31, 26.44, 24.47. HRMS–ESI (m/z): calcd for C₁₇H₂₃NO₄ ([M + H]⁺), 306.1661; found, 306.1648.

4e, Pale yellow oil, 80% yield (215 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.66 (m, 2H), 7.32 (m, 2H), 7.24 (m, 3H), 6.88 (d, *J* = 8.11 Hz, 2H), 3.70 (m, 2H), 3.04 (s, 5H), 2.92 (t, *J* = 6.87 Hz, 2H). ¹³C NMR (101 MHz, MeOD) δ 170.31, 170.24, 154.07, 140.69, 129.81, 129.65, 129.41, 127.23, 121.94, 112.06, 47.71, 42.54, 40.20, 36.79, 9.17. HRMS–ESI (m/z): calcd for C₁₇H₂₀N₂O ([M + H]⁺), 269.1609; found, 269.1590.

4f, Pale yellow solid, 52% yield (139 mg). ¹H NMR (400 MHz, CDCl₃) δ 7.69 (m, 2H), 7.37 (m, 4H), 7.27 (m, 1H), 6.68 (m, 2H), 6.21 (m, 1H), 5.33 (p, *J* = 7.05 Hz, 1H), 3.01 (s, 6H), 1.59 (d, *J* = 6.91 Hz, 3H). ¹³C NMR (101 MHz, MeOD) δ 169.70, 154.24, 145.78, 129.88,

129.42, 127.86, 127.14, 122.08, 112.10, 50.37, 40.23, 22.40. HRMS–ESI (m/z): calcd for $C_{17}H_{20}N_2O$ ($[M + H]^+$), 269.1609; found, 269.1583.

4g, Yellow oil, 67% yield (138 mg). 1H NMR (400 MHz, $CDCl_3$) δ 7.65 (m, 2H), 6.65 (m, 2H), 5.82 (d, $J = 7.24$ Hz, 1H), 4.27 (m, 1H), 4.20 (s, 1H), 3.06 (m, 2H), 3.00 (s, 6H), 1.39 (t, $J = 7.32$ Hz, 2H), 1.36 (s, 0H), 1.23 (d, $J = 6.54$ Hz, 6H). ^{13}C NMR (101 MHz, MeOD) δ 169.59, 154.04, 129.73, 122.55, 112.21, 112.11, 47.82, 42.79, 40.35, 22.73, 9.20. HRMS–ESI (m/z): calcd for $C_{12}H_{18}N_2O$ ($[M + H]^+$), 207.1453; found, 207.1438.

4h, Yellow oil, 71% yield (165 mg). 1H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 2H), 6.31 (d, $J = 8.41$ Hz, 2H), 5.57 (d, $J = 6.28$ Hz, 1H), 3.99 (q, $J = 6.94$ Hz, 1H), 2.62 (s, 6H), 1.68 (m, 2H), 1.28 (m, 3H), 1.08 (dddd, $J = 1.47, 6.78, 11.92, 13.46$ Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 170.23, 154.12, 129.75, 122.31, 112.07, 52.82, 40.25, 33.52, 24.91. HRMS–ESI (m/z): calcd for $C_{14}H_{20}N_2O$ ($[M + H]^+$), 233.1609; found, 233.1589.

4i, Pale yellow solid, 55% yield (156 mg). 1H NMR (400 MHz, MeOD) δ 7.83 (m, 2H), 7.46 (m, 2H), 6.79 (m, 4H), 3.04 (s, 6H), 2.92 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 168.82, 154.31, 149.59, 130.02, 124.06, 122.47, 114.50, 112.16, 41.43, 40.25. HRMS–ESI (m/z): calcd for $C_{17}H_{21}N_3O$ ($[M + H]^+$), 284.1718; found, 284.1617.

4j, Pale yellow solid, 62% yield (158 mg). 1H NMR (400 MHz, $CDCl_3$) δ 7.63 (m, 2H), 7.25 (m, 4H), 7.19 (m, 1H), 6.67 (d, $J = 8.49$ Hz, 2H), 6.29 (s, 1H), 4.54 (m, 2H), 3.31 (s, 1H), 2.93 (s, 6H). ^{13}C NMR (101 MHz, MeOD) δ 170.30, 154.16, 140.67, 129.83, 129.45, 128.55, 128.40, 127.99, 122.11, 112.31, 44.32, 40.35, 9.21. HRMS–ESI (m/z): calcd for $C_{16}H_{18}N_2O$ ($[M + H]^+$), 255.1453; found, 255.1454.

4k, Pale yellow solid, 71% yield (206 mg). 1H NMR (400 MHz, MeOD) δ 7.92 (dd, $J = 3.47, 9.22$ Hz, 3H), 7.83 (m, 2H), 7.73 (d, $J = 8.13$ Hz, 1H), 7.44 (m, 4H), 6.69 (d, $J = 8.64$ Hz, 2H), 2.93 (s, 6H), 1.73 (s, 0H), 1.64 (s, 0H), 0.99 (m, 0H). ^{13}C NMR (101 MHz, MeOD) δ 169.82, 154.28, 135.67, 134.96, 133.41, 131.06, 130.39, 129.24, 127.78, 127.11, 127.03, 126.47, 125.07, 124.01, 121.69, 112.20, 112.00, 111.80, 40.25, 40.03, 40.01. HRMS–ESI (m/z): calcd for $C_{19}H_{18}N_2O$ ($[M + H]^+$), 291.1453; found, 291.1434.

4l, Pale yellow solid, 70% yield (193 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.27 (d, $J = 8.64$ Hz, 2H), 6.37 (d, $J = 8.64$ Hz, 2H), 2.98 (m, 2H), 2.60 (s, 6H), 1.16 (ddd, $J = 6.37, 8.00, 14.68$ Hz, 2H), 0.92 (m, 2H), 0.85 (m, 12H), 0.45 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 170.17, 170.09, 153.96, 129.66, 122.15, 112.03, 41.20, 40.90, 40.27, 32.97, 32.90, 30.74, 30.48, 30.45, 30.40, 30.31, 30.24, 30.15, 28.16, 27.67, 23.69, 23.66, 14.53, 14.50. HRMS–ESI (m/z): calcd for $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}$ ($[\text{M} + \text{H}]^+$), 277.22235; found, 277.2236.

4m, Pale yellow oil, 90% yield (270 mg). ^1H NMR (400 MHz, MeOD) δ 7.72 (m, 2H), 6.67 (m, 2H), 5.28 (tq, $J = 1.35, 6.73$ Hz, 1H), 5.07 (dddt, $J = 1.35, 3.00, 5.88, 7.08$ Hz, 1H), 4.87 (s, 2H), 3.95 (d, $J = 6.82$ Hz, 2H), 2.93 (s, 6H), 2.02 (m, 5H), 1.63 (m, 10H). ^{13}C NMR (101 MHz, MeOD) δ 169.21, 153.72, 139.09, 132.24, 129.04, 125.55, 122.18, 112.26, 40.41, 38.61, 27.55, 25.95, 18.37, 16.44. HRMS–ESI (m/z): calcd for $\text{C}_{19}\text{H}_{28}\text{N}_2\text{O}$ ($[\text{M} + \text{H}]^+$), 301.2235; found, 301.2215.

4n, Pale yellow solid, 27% yield (71 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.73 (m, 2H), 6.75 (d, $J = 8.46$ Hz, 2H), 6.49 (d, $J = 8.65$ Hz, 1H), 3.76 (s, 3H), 3.06 (d, $J = 8.36$ Hz, 2H), 3.03 (s, 6H), 0.99 (dd, $J = 6.87, 7.85$ Hz, 6H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.42, 167.75, 133.52, 128.28, 112.47, 110.99, 58.39, 53.31, 41.51, 40.20, 29.92, 22.05, 18.14. HRMS–ESI (m/z): calcd for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_3$ ($[\text{M} + \text{H}]^+$), 265.1507; found, 265.1509.

4o, Pale yellow oil, 87% yield (207 mg). ^1H NMR (400 MHz, CDCl_3) δ 7.70 (d, $J = 2.36$ Hz, 1H), 7.27 (dd, $J = 1.83, 7.85$ Hz, 3H), 7.03 (s, 1H), 6.96 (dd, $J = 1.97, 3.94$ Hz, 1H), 6.94 (q, $J = 1.83, 2.37$ Hz, 1H), 6.90 (m, 1H), 2.07 (t, $J = 7.31$ Hz, 2H), 1.85 (td, $J = 2.70, 6.76$ Hz, 2H), 1.51 (m, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.91, 135.34, 133.95, 130.76, 128.89, 127.77, 127.67, 126.66, 125.16, 119.94, 116.78, 83.58, 69.61, 36.17, 24.08, 17.95. HRMS–ESI (m/z): calcd for $\text{C}_{16}\text{H}_{15}\text{NO}$ ($[\text{M} + \text{H}]^+$), 238.1187; found, 238.1172.

6, White solid, 90% yield (426 mg). ^1H NMR (400 MHz, CDCl_3) δ 5.31 (d, $J = 8.22$ Hz, 1H), 3.85 (t, $J = 3.03$ Hz, 1H), 3.75 (dtt, $J = 4.36, 7.90, 11.19$ Hz, 1H), 3.48 (s, 1H), 3.45 (dq, $J = 4.42, 5.59, 11.01$ Hz, 1H), 2.20 (m, 2H), 2.14 (s, 7H), 2.03 (m, 1H), 1.94 (m, 3H), 1.88 (m, 1H), 1.83 (m, 1H), 1.79 (dd, $J = 2.90, 5.65$ Hz, 1H), 1.66 (m, 4H), 1.43 (m, 6H), 1.26 (m, 1H), 1.13 (m, 6H), 0.93 (m, 8H), 0.65 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 175.67, 72.81, 68.96, 57.32, 51.51, 51.35, 49.85, 49.58, 43.66, 43.16, 41.04, 40.76, 40.44, 36.88, 36.58, 36.20, 36.04, 35.92, 35.10, 34.22, 34.02, 33.85, 33.77, 33.43, 31.35, 29.29, 26.66, 26.45,

26.18, 25.92, 24.62, 23.45, 23.25, 22.09, 21.79, 18.98, 12.23. HRMS–ESI (m/z): calcd for $C_{30}H_{51}NO_3$ ([M + H]⁺), 474.3902; found, 474.3892.

7a, Pale yellow solid, 53% yield (221 mg). ¹H NMR (400 MHz, CDCl₃) δ 8.14 (s, 1H), 7.66 (d, *J* = 7.84 Hz, 1H), 7.35 (m, 1H), 7.19 (ddd, *J* = 1.25, 6.99, 8.07 Hz, 1H), 7.12 (ddd, *J* = 1.10, 6.97, 8.01 Hz, 1H), 7.09 (s, 1H), 6.30 (d, *J* = 8.47 Hz, 1H), 5.19 (s, 1H), 4.45 (s, 1H), 4.40 (dd, *J* = 5.01, 8.50 Hz, 1H), 3.63 (s, 3H), 3.29 (dd, *J* = 5.82, 14.86 Hz, 1H), 3.19 (dd, *J* = 7.47, 14.57 Hz, 1H), 2.02 (qd, *J* = 5.08, 7.00 Hz, 1H), 1.43 (s, 9H), 1.18 (m, 1H), 0.79 (dd, *J* = 6.87, 11.15 Hz, 6H). ¹³C NMR (101 MHz, CDCl₃) δ 171.76, 171.71, 155.56, 136.28, 127.49, 123.31, 122.17, 119.69, 118.83, 111.19, 110.56, 80.14, 57.31, 55.34, 52.03, 40.91, 31.23, 28.31, 28.11, 23.90, 18.72, 17.76, 17.53, 17.32, 14.67, 7.95. HRMS–ESI (m/z): calcd for $C_{23}H_{32}N_2O_5$ ([M + H]⁺), 417.2345; found, 417.2343.

7b, Pale yellow solid, 70% yield (275 mg). Synthesis performed from racemic N-Boc Hydroxyproline. The product is obtained as a mixture of diastereomers. ¹H NMR (400 MHz, CDCl₃) δ 7.29 (dd, *J* = 6.98, 13.65 Hz, 3H), 7.16 (d, *J* = 7.21 Hz, 2H), 6.78 (s, 0H), 4.87 (m, 1H), 4.68 (s, 2H), 4.37 (s, 2H), 3.73 (s, 3H), 3.58 (m, 1H), 3.45 (s, 0H), 3.42 (s, 2H), 3.20 (dd, *J* = 5.83, 13.85 Hz, 1H), 3.06 (dd, *J* = 7.09, 13.89 Hz, 1H), 2.27 (m, 1H), 1.83 (m, 1H), 1.46 (m, 10H). ¹³C NMR (101 MHz, CDCl₃) δ 175.44, 174.39, 172.63, 171.92, 171.70, 155.70, 155.35, 154.94, 154.28, 135.87, 129.14, 128.46, 127.04, 81.02, 80.66, 80.46, 69.49, 68.98, 59.70, 58.65, 57.89, 57.66, 54.75, 54.60, 54.42, 53.32, 52.86, 52.25, 50.15, 39.29, 38.94, 37.89, 37.68, 37.01, 28.34, 28.26, 28.18, 28.07, 23.89, 22.86. HRMS–ESI (m/z): calcd for $C_{20}H_{28}N_2O_6$ ([M + H]⁺), 393.1981; found, 393.2005.

7c, Pale yellow solid, 62% yield (218 mg). ¹H NMR (400 MHz, CDCl₃) δ 6.91 (d, *J* = 8.42 Hz, 3H), 6.74 (d, *J* = 7.53 Hz, 1H), 6.71 (s, 1H), 6.68 (s, 1H), 5.30 (s, 1H), 4.82 (dt, *J* = 5.74, 8.27 Hz, 1H), 3.72 (s, 4H), 3.70 (s, 0H), 3.01 (qd, *J* = 5.76, 14.03 Hz, 2H), 1.44 (s, 9H). ¹³C NMR (101 MHz, CDCl₃) δ 172.17, 169.66, 156.33, 155.67, 130.43, 126.90, 115.78, 80.68, 53.40, 52.61, 44.21, 37.17, 28.40. HRMS–ESI (m/z): calcd for $C_{17}H_{24}N_2O_6$ ([M + H]⁺), 353.1668; found, 353.1642.

7d, Pale yellow solid, 28% yield (107 mg). ¹H NMR (400 MHz, CDCl₃) δ 8.64 (s, 2H), 7.69 (d, *J* = 7.30 Hz, 1H), 7.58 (s, 1H), 6.81 (s, 1H), 5.24 (d, *J* = 7.40 Hz, 1H), 4.68 (q, *J* = 6.13 Hz, 1H), 4.12 (s, 1H), 3.66 (s, 3H), 3.12 (q, *J* = 6.27 Hz, 2H), 1.63 (m, 2H), 1.47 (m, 1H),

1.40 (s, 10H), 1.21 (d, $J = 16.17$ Hz, 1H), 1.20 (s, 1H), 0.90 (m, 7H). ^{13}C NMR (101 MHz, CDCl_3) δ 172.90, 171.70, 156.15, 135.20, 131.83, 118.47, 80.40, 53.64, 52.74, 52.55, 41.27, 29.77, 28.41, 24.84, 23.06, 21.86. HRMS–ESI (m/z): calcd for $\text{C}_{18}\text{H}_{30}\text{N}_4\text{O}_5$ ($[\text{M} + \text{H}]^+$), 383.2250; found, 383.2240.

High scale synthesis of N-cyclohexyl-4-(dimethylamino)benzamide **3**

In a 20 mL microwave reaction vial were introduced in 15 mL of dichloromethane, 3.22 g of dimethylamino benzoic acid **1** (20 mmol), 2.8 g of cyclohexylamine **2** (28 mmol), 2.8 g of NEt_3 (28 mmol) and 1.27 g (1.2 mL) of T3P (50 wt.% in ethylacetate) (28 mmol). Microwave reaction vial was sealed with a cap and a septum. Reaction was performed under microwave irradiations (450 Watt) at 120 °C for 20 minutes using a normal mode and a pre-stirring of 20 seconds. The organic phase was then washed twice with 15 mL of 10% NaHCO_3 solution. The aqueous phases were extracted with 3x 10 mL of dichloromethane, and the combined organic phases were dried over Na_2SO_4 , filtered and concentrated *in vacuo* to afford the expected product N-cyclohexyl-4-(dimethylamino)benzamide **3** as a yellow oil in 81% yield (3.9 g).

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