Application of Polymer-Supported Enzymes and Reagents in the Synthesis of γ-Aminobutyric Acid (GABA) Analogues

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Abstract: Polymer-supported pig liver esterase was used for the resolution of *meso*-diesters. The enzyme can be recovered quantitatively from the reaction mixture by filtration and reused without significant loss of activity. Further transformation of the resulting enantiomerically enriched carboxylic acids through the application of polymer-supported reagents and scavengers provides a number of GABA-analogues.

Key words: polymer-supported enzymes, pig liver esterase, polymer-supported reagents, GABA-analogues, desymmetrisation

Enzymes have become valuable tools in asymmetric organic synthesis, notably in the desymmetrisation of *meso*-compounds.[1] In this way, achiral starting materials can be converted into enantiomerically enriched compounds in up to quantitative yield. However, the high cost of enzyme preparations have made recycling of the biocatalyst an important issue. Since filtration is a simple way of recovering a catalyst from a reaction mixture, enzymes have been covalently linked to a multitude of insoluble supports and adsorbed onto various materials. However, immobilising enzymes can modify their properties and care has to be taken not to alter the selectivity and the activity of the native catalyst.[2]

With the need for automated parallel synthesis of large numbers of pharmaceutically interesting compounds, polymer-supported reagents have been developed that display high degree of versatility and applicability in recent years.[3] Compared to traditional solution phase and solid phase chemistry, supported reagents offer many advantages for example the reactions can be monitored by conventional techniques (TLC, LC-MS, GC, NMR) and no additional steps are required in order to attach and subsequently remove the substrate from the support. As yet, polymer-supported enzymes have not been effectively integrated into the toolbox of reagents for multistep synthesis.

Analogues of the neurotransmitter γ-aminobutyric acid (GABA) have been used to explore the binding mode and structure-activity relationship of the parent compound.[4] They have also been investigated as therapeutic agents for a range of central nervous system disorders.[5] Specific conformations of GABA can be mimicked using conformationally locked compounds containing a rigid backbone.[6] Here we wish to report the first combined application of polymer-supported enzymes and reagents in the synthesis of structurally defined GABA-analogues.

The initial step of our synthetic route involved the desymmetrisation of commercially available *meso*-diester **1** with polymer-supported pig liver esterase on Eupergit® (Scheme 1).[7] The reaction was carried out in an aqueous phosphate buffer system, which was kept at pH 7 by continuous addition of 0.5 N NaOH by a pH stat. After completion of the reaction, the enzyme was removed by filtration and reused in subsequent runs. We noticed an increase in the rate of hydrolysis with each successive cycle of the enzyme. The reaction required 26 hours to reach completion when the enzyme was used for the first time, only 12 hours in the second cycle and 9 hours in the third cycle. This was probably due to a preadjustment of conformation of the enzyme by incorporation of water molecules after reaction. In every cycle the hydrolysis proceeded without any decrease of the enantiomeric excess of the product. The desired carboxylic acid **2** was isolated in 90% ee from the acidified reaction mixture (saturated with sodium chloride).[8] The enantiomeric excess could be increased to >95% ee by twofold recrystallisation from benzene.

Reduction of the carboxylic acid **2** to **3** and **4** was achieved using $BH_3{\cdot}SMe_2$ in THF.[9] This reaction was quenched with a small amount of water and the resulting boric acid scavenged using the boron specific resin Amberlite IRA-743.[10] The ratio of **3** and **4** depended on the excess of $BH_3{\cdot}SMe_2$ used. Any attempts to apply polymer-supported reducing agents in this reaction failed. Both alcohol **3** and lactone **4** were transformed into bromide **5** using an excess of thionyl bromide in methanol. Neutralisation was achieved by addition of poly(4-vinylpyridine)[11] to the reaction mixture. The conversion of bromide **5** into azide **6** proceeded smoothly in 84% yield by application of polymer-supported azide[12] and iodide[13] in acetonitrile. Reduction of azide **6** using either Staudinger conditions involving polymer-supported triphenylphosphine[14] or Pd/C under a hydrogen atmosphere furnished a mixture of the desired amino acid ester and the corresponding lactam. To suppress lactamisation, **6** was reacted with Pd/C and Boc-anhydride under hydrogen atmosphere to convert the amine in situ into the Boc-protected derivative **7**. Excess Boc_2O was scavenged using an amino resin.[15] Finally, application of a catch and release technique for

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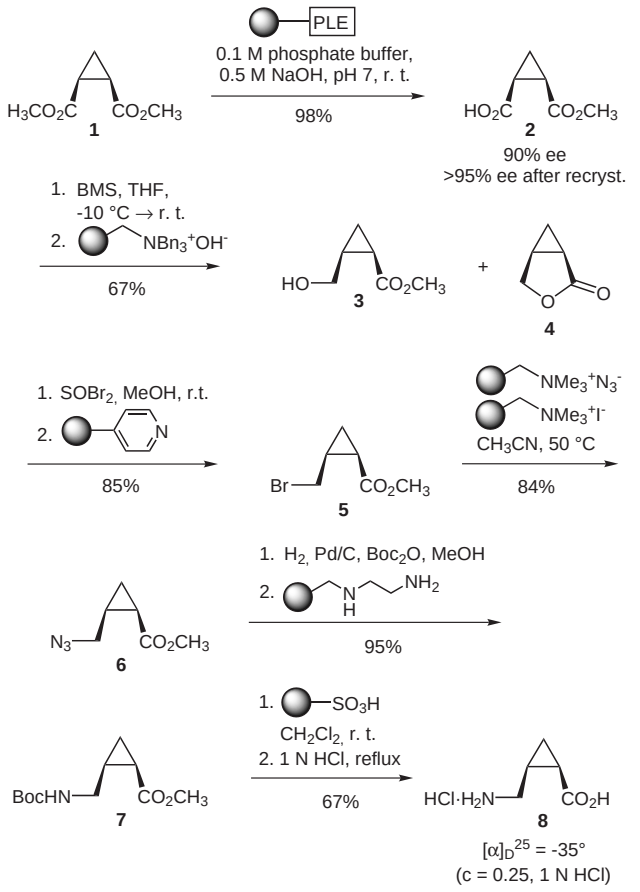
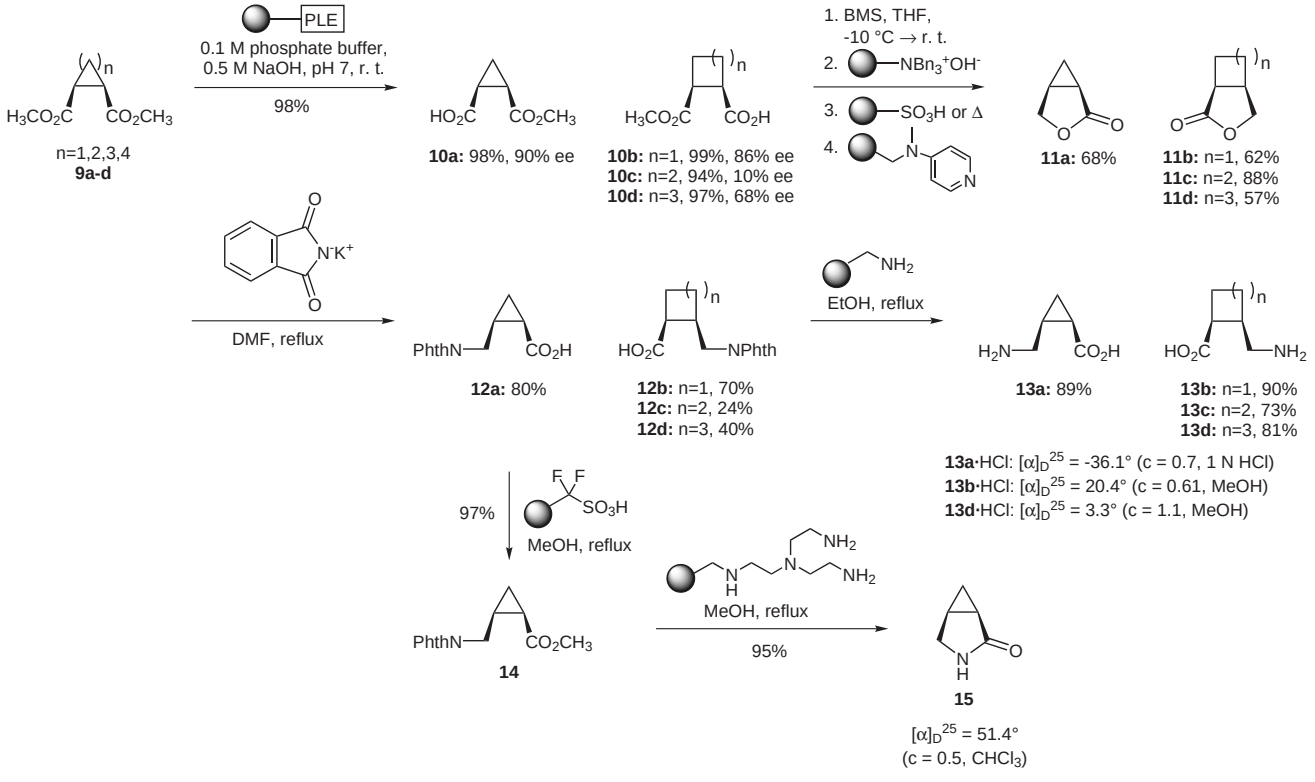


Scheme 1 Synthesis of GABA-analogue **8** with polymer-supported reagents and enzymes.

removal of the protecting group and saponification of the methyl ester using Amberlyst A-15 and HCl gave the GABA-analogue **8** as its hydrochloride in 67% yield.[16]

The presented reaction sequence affords the desired product without the requirement of any column chromatography and therefore demonstrates clearly the advantages of polymer-supported enzymes and reagents. We next expanded this reaction sequence to analogues with a four, five and six membered ring. The synthetic strategy was altered since these analogues are more conveniently prepared using a combination of polymer-supported and traditional solution phase chemistry.

Starting from *meso*-diesters **9a**–**d** the enzymatic hydrolysis of these compounds proceeded smoothly and the enzyme could be reused after its isolation by filtration (Scheme 2). The enantiomeric ratios are similar to those observed with the corresponding non-immobilized enzyme.[17] *Meso*-cyclopentane dicarboxylic acid dimethyl ester **9c** and *meso*-cyclohexane dicarboxylic acid dimethyl ester **9d** proved to be poor substrates for the polymer-supported pig liver esterase providing enantiomeric excesses of 10% and 68% respectively. The enatiomeric ratios were determined by ^{1}H NMR analysis of the corresponding salt formed with (+)-(*R*)-α-methylbenzylamine. We were able to confirm the absolute stereochemistry of **10a** by a X-ray structure of the corresponding diastereomeric amide formed from **12a** and (+)-(*R*)-α-methylbenzylamine.[18] In case of **10b** the absolute stereochemistry was assigned by comparison of the optical rotations of **10b** and **13b** with literature values.[19] The



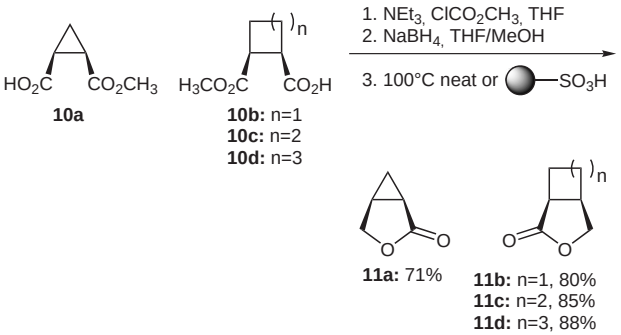
Scheme 2 Synthesis of GABA-analogues **13a**–**d** with polymer-supported reagents and enzymes.

measured rotations for **10b** and **13b** indicate a reversal of the selectivity of the immobilized enzyme in contrast to the non-immobilized pig liver esterase.

The reduction of the carboxylic acid function was achieved using the same procedure as for compound **2**. The resulting mixture of lactone and corresponding alcohol was transformed into the pure lactones **11a–d** by heating the mixture neat or by reaction with Amberlyst A-15 in CH_2Cl_2. Remaining non-reduced carboxylic acids **10a–d** could be scavenged with polymer-supported DMAP. A small amount of impurity in this reaction was responsible for the formation of side products in the following lactone ring-opening step and therefore made chromatographic purification of the resulting carboxylic acids **12a–d** necessary (for an alternative approach see below). The opening of the lactone ring in **11a–d** was achieved by reaction with potassium phthalimide in refluxing DMF.[6a] This reaction proceeded smoothly in the case of the GABA-analogues with a three and a four membered ring **12a** and **12b** due to the strained ring system. In the case of the five membered ring **12c** the yield is poor and epimerisation at the α-position of the carboxylic acid occurs due to the long reaction time (24 h).[20] All carboxylic acids were isolated by an aqueous basic-acidic work-up sequence. The phthalimide protecting group could be removed by refluxing **12a–d** with aminomethylated polystyrene resin[21] in EtOH. GABA-analogues **13a–d** were obtained after hot filtration, washing and evaporation in 73–90% yield.[22] Other polymer-supported amines and hydrazines did not react or resulted in scavenging of the starting material. In contrast, deprotection of the amino group of methyl ester **14** (obtained from **12a** by acid catalysed esterification) with trisamine resin[23] furnished lactam **15** selectively.[24]

As mentioned above, the reduction of the carboxylic acids **10a–d** was usually accompanied by the formation of an impurity, which caused purification problems in the following ring-opening step. To circumvent this problem, we developed a reaction sequence, which furnishes the lactones **11a–d** in good yields and purity without costly purification (Scheme 3). Acids **10a–d** were transformed into their corresponding ethyl carbonates, which were reduced using $NaBH_4$ in THF by slow addition of a small amount of MeOH.[25] After evaporation, the crude products were redissolved again in a petrol ether/ethyl acetate mixture (1:1) and filtered through a short plug of silica. The alcohols obtained were transformed into the lactones **11a–d** by acid catalysis or by heating them neat to 100 °C.

The short syntheses of GABA-analogues shown above represent an useful application of polymer-supported reagents. The advantages of this strategy are not only the circumvention of classical purification methods like column chromatography and distillation, but also the introduction of chirality by use of polymer-supported enzymes. This type of polymer-supported reagent has to date not been employed in a polymer-supported reaction sequence and represents an extension to the toolbox of available reagents for potential automated syntheses.



Scheme 3 Improved synthesis of lactones **11a–d**.

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(7) Purchased from Fluka Cat. No. 46064; solid support: methacrylamide/allyl glycidylether/methylene-bis-acrylamide; loading: ~500 U/g.
(8) Enantiomeric ratio of **2** using non-immobilized pig liver esterase: 100% ee.[1a]
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(10) Purchased from Aldrich Cat. No. 21,644-5.
(11) Purchased from Fluka Cat. No. 81393; solid support: poly(4-vinylpyridine)/25% DVB.
(12) Purchased from Aldrich Cat. No. 36,834-2; solid support: styrene/DVB; loading: 3.8 mmol/g.
(13) Purchased from Fluka Cat. No. 57895; solid support: styrene/>20% DVB; loading: 2.9 mmol/g.
(14) Purchased from Fluka Cat. No. 93093; solid support: styrene/2% DVB; loading: 3 mmol/g.
(15) Purchased from Novabiochem Cat. No. 01-64-0178; solid support: styrene/1% DVB; loading 2.0–3.5 mmol/g.
(16) Lit. value **8**: $[\alpha]_D^{25}$ = –38.1 (*c* 1, 1 N HCl).[6d]
(17) Enantiomeric ratios of **10a**–**d** using non-immobilized pig liver esterase: **10a**: 100% ee, **10b**: 90% ee, **10b**: 9% ee, **10c**: 78% ee.[1a]
(18) For the observed inconsistencies compare reported optical rotation in references 1a and 1c. We wish to report the following rotation **10a**: $[\alpha]_D^{25}$ = –17.4 (*c* 0.8, $CHCl_3$). Crystallographic data of the amide formed from **12a** and (+)-(*R*)-α-methylbenzylamine have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC-189257. 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; deposit@ccdc.cam.ac.uk).
(19) For compound **10b** we measured $[\alpha]_D^{25}$ = –3.6 (*c* 0.95, $CHCl_3$), which is the opposite of the rotations published in references 1a,c.
(20) According to the ^{13}C NMR spectrum the ratio of the diastereomers is 1:1.7.
(21) Purchased from Novabiochem Cat. No. 01-64-0177; solid support: styrene/2% DVB; loading: 2.0–3.0 mmol/g.
(22) Lit. values: **13a·HCl**: $[\alpha]_D^{25}$ = –38.1 (*c* 1, 1 N HCl);[6d] **13b·HCl**: $[\alpha]_D^{25}$ = 24.1 (*c* 0.4, MeOH);[6h] analytical data for **13d**: ^1H NMR (400 MHz, D_2O): δ = 1.25–1.71 (m, 8 H), 2.08–2.19 (m, 1 H), 2.43–2.50 (m, 1 H), 2.97 (dd, *J* = 12.8, 5.9 Hz, 1 H), 3.05 (dd, *J* = 12.8, 7.7 Hz, 1 H); ^{13}C NMR (100 MHz, D_2O): δ = 21.9, 24.5, 26.5, 27.3, 35.6, 40.6, 47.6, 183.6; **13d·HCl**: $[\alpha]_D^{25}$ = 3.3 (*c* 1.1, MeOH).
(23) Purchased from Novabiochem Cat. No. 01-64-0170; solid support: styrene/1% DVB; loading: 2.2 mmol/g.
(24) Lit. value **15**: $[\alpha]_D^{25}$ = 49.2 (*c* 1, $CHCl_3$).[6h]
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