Article

Stereoselective Bioreduction of α-diazo-β-keto Esters

Sergio González-Granda 1, Taissa A. Costin 2, Marcus M. Sá 2,* and Vicente Gotor-Fernández 1,*

1 Organic and Inorganic Chemistry Department, University of Oviedo, Avenida Julián Clavería 8, Oviedo 33006, Spain; sergioglezgranda@gmail.com (S.G.-G.)
2 Chemistry Department, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil; taissacostin@yahoo.com.br (T.A.C.)
* Correspondence: marcus.sa@ufsc.br (M.M.S.); vicgotfer@uniovi.es (V.G.-F.)

Academic Editor: Paola Vitale

Received: 30 January 2020; Accepted: 14 February 2020; Published: 19 February 2020

Abstract: Diazot compounds are versatile reagents in chemical synthesis and biology due to the tunable reactivity of the diazo functionality and its compatibility with living systems. Much effort has been made in recent years to explore their accessibility and synthetic potential; however, their preparation through stereoselective enzymatic asymmetric synthesis has been scarcely reported in the literature. Alcohol dehydrogenases (ADHs, also called ketoreductases, KREDs) are powerful redox enzymes able to reduce carbonyl compounds in a highly stereoselective manner. Herein, we have developed the synthesis and subsequent bioreduction of nine α-diazo-β-keto esters to give optically active α-diazo-β-hydroxy esters with potential applications as chiral building blocks in chemical synthesis. Therefore, the syntheses of prochiral α-diazo-β-keto esters bearing different substitution patterns at the adjacent position of the ketone group (N3CH2, CICH2, BrCH2, CH3OCH2, NCSCH2, CH3, and Ph) and in the alkyoxy portion of the ester functionality (Me, Et, and Bn) were carried out through the diazo transfer reaction to the corresponding β-keto esters in good to excellent yields (81–96%). After performing the chemical reduction of α-diazo-β-keto esters with sodium borohydride and developing robust analytical conditions to monitor the biotransformations, their bioreductions were exhaustively studied using in-house made Escherichia coli overexpressed and commercially available KREDs. Remarkably, the corresponding α-diazo-β-hydroxy esters were obtained in moderate to excellent conversions (60 to >99%) and high selectivities (85 to >99% ee) after 24 h at 30 °C. The best biotransformations in terms of conversion and enantiomeric excess were successfully scaled up to give the expected chiral alcohols with almost the same activity and selectivity values observed in the enzyme screening experiments.

Keywords: alcohol dehydrogenases; asymmetric synthesis; bioreduction; diazo compounds; enzymes; hydroxy esters

1. Introduction

α-Diazo carbonyl compounds are widely recognized as versatile reagents in organic synthesis and chemical biology [1–5]. The chemistry of α-diazo-β-hydroxy carbonyl compounds attracts particular attention, as these functionalized compounds have been employed in the synthesis of amino acid analogues and heterocycles of biological relevance [6–11]. The conventional preparation of α-diazo-β-hydroxy carbonyl compounds relies on the aldol-type addition of a terminal diazo carbonyl compound with aldehydes or ketones mediated by a strong base such as the organometallics of lithium, magnesium, or zinc, DBU, and NaOH [12–16]. Asymmetric versions of the aldol reaction involving α-diazo esters and aldehydes have been reported by several authors [1,2,17,18], but the best yields and enantioselectivities were achieved by Trost and co-workers using a dinuclear magnesium complex [19,20]. Although each method possesses its own advantages, the use
Molecules 2020, 25, 931

of basic medium generally requires strictly anhydrous conditions and cryogenic temperatures, not mentioning the incompatibility of more labile functional groups, thereby severely limiting the scope of these synthetic methodologies.

An alternative approach to accessing α-diazo-β-hydroxy esters consists of the chemoselective carbonyl reduction of the corresponding α-diazo-β-keto esters, although this strategy has only been applied occasionally [11,21,22]. Interestingly, the asymmetric reduction of α-diazo-β-keto esters to the corresponding chiral α-diazo-β-hydroxy esters has not been described so far, even though the enzyme-catalyzed reduction of carbonyl compounds to secondary alcohols is one of the most reliable methods to deliver a wide variety of chiral alcohols under mild conditions [23–28].

The use of enzymes, and particularly alcohol dehydrogenases (ADHs, KREDs), as sustainable catalysts is a powerful synthetic tool for both academy and industry since they are non-toxic, biodegradable, and display exquisite chemo, regio, and stereoselectivity in synthetic transformations [29–32]. In this context, remarkable chemoenzymatic transformations involving diazo compounds have been described in the past years [33–35]. However, the asymmetric reduction of ketones to alcohols in the presence of the diazo group had been completely neglected until very recently [36].

Taking into account the importance of α-diazo-β-hydroxy esters in synthetic chemistry and chemical biology, and motivated by the absence of studies dealing with the asymmetric reduction of α-diazo carbonyl compounds, herein is presented the enantioselective synthesis of γ-functionalized α-diazo-β-hydroxy esters through the bioreduction of readily available α-diazo-β-keto esters by focusing on the screening of ketoreductases as versatile stereoselective biocatalysts of wide applicability.

2. Results and Discussion

2.1. Chemical Synthesis of α-Diazo-β-keto Esters 2a–i and Racemic α-Diazo-β-hydroxy Esters 3a–i

The investigation commenced with the racemic synthesis of representative α-diazo-β-hydroxy esters 3a–i through the chemical reduction of selected α-diazo-β-keto esters 2a–i with different substitution patterns (Scheme 1) in order to develop adequate analytical methods for the measurement of conversion and enantiomeric excess of the optically active alcohols obtained from bioreduction experiments (see below). Firstly, the amine-catalyzed diazo transfer reaction developed by Sá and cols [37] was the method of choice to prepare diazo compounds 2 due to its high efficiency and broad substrate scope. Therefore, the preparation of the starting α-diazo-β-keto esters 2b–i was readily achieved in 81–96% yield (3–5 mmol scale) through the diazo transfer protocol from 4-acetamidobenzenesulfonyl azide (p-ABSA) and the corresponding β-keto esters 1b–i (right side of Scheme 1 and Table 1), which are commercially available or can be easily prepared from simple reagents (see the Experimental section). In the particular case of ethyl 4-azido-2-diazo-3-oxobutanoate (2a), this versatile building block was obtained in 86% yield following the one-pot procedure from ethyl 4-chloroacetacetate (1b) to introduce both the azido and diazo functionalities as previously developed by Sá and cols (left side of Scheme 1 and Table 1) [37].
Scheme 1. Chemical synthesis of α-diazo-β-keto esters 2a–i and their reduction to α-diazo-β-hydroxy esters 3a–i.

Next, diazo ketone esters 2a–i were chemically reduced by employing sodium borohydride (NaBH₄) in tetrahydrofuran (THF) as the solvent at 0 °C for only 10 min to give the expected α-diazo-β-hydroxy esters 3a–i in moderate to high yields after column chromatography purification (62–85%, Table 1).

| Entry | Compound | R¹ | R² | Keto ester 2 (%) | Hydroxy ester 3 (%) |
|-------|----------|----|----|-----------------|-------------------|
| 1     | a        | N(CH₂)₂ | CH₃CH₂ | 86 b          | 71                |
| 2     | b        | CICH₂  | CH₃CH₂ | 96 b          | 75                |
| 3     | c        | CICH₂  | CH₃  | 90            | 69                |
| 4     | d        | CH₃    | CH₃  | 81            | 62                |
| 5     | e        | CH₃OCH₂ | CH₃  | 92            | 78                |
| 6     | f        | C₆H₅    | CH₃CH₂ | 95            | 85                |
| 7     | g        | CH₃    | C₆H₅CH₂ | 90          | 78                |
| 8     | h        | BrCH₂  | CH₃CH₂ | 94            | 71                |
| 9     | i        | NCSCH₂ | CH₃CH₂ | 88          | 77                |

* Isolated yields after column chromatography on silica gel.

b Compounds 2a and 2b were both obtained from ethyl 4-chloroacetate (1b).

With α-diazo-β-keto esters 2a–i and racemic α-diazo-β-hydroxy esters 3a–i in hand, robust analytical methods were developed for the determination of the conversion values in the corresponding bioreduction experiments and the measurements of the optical purity of 3a–i through the selection of HPLC techniques and a series of HPLC chiral columns (see Tables S1–S10 in the Supplementary Material).

2.2. Bioreduction of α-Diazo-β-keto Esters 3a–i using Alcohol Dehydrogenases

2.2.1. Bioreduction of Ethyl 4-Azido-2-diazo-3-oxobutanoate (2a)

Ethyl 4-azido-2-diazo-3-oxobutanoate (2a) was selected as the model substrate to find active alcohol dehydrogenases (ADHs) that would be able to selectively furnish the chiral alcohol 3a through the chemoselective bioreduction of the keto group. Therefore, a representative group of in-house made Escherichia coli overexpressed ADHs was evaluated (Table 2), including the enzymes from Rhodococcus ruber (ADH-A) [38], Thermoanaerobium species (ADH-T) [39], Lactobacillus brevis (LB-ADH) [40,41], Ralstonia species (Ras-ADH) [42,43], Sphingobium yanoikuyae (Sy-ADH), [44,45], and Thermoanaerobacter ethanolicus (Tes-ADH) [46]. Unfortunately, poor activities (<10% conversion, entries 1-6) were observed for all these E. coli overexpressed ADHs, except for the Ras-ADH, which led to the formation of a complex mixture of unidentified products [47].
Table 2. Bioreduction of ethyl 4-azido-2-diazo-3-oxobutanoate (2a) using different ADHs.

![Chemical structure](image_url)

| Entry | ADH/KRED          | Conversion (%) | Alcohol 3a ee (%) |
|-------|-------------------|----------------|------------------|
| 1     | Ras-ADH           | n.m.           | -                |
| 2     | LB-ADH            | <5             | -                |
| 3     | Sy-ADH            | 6              | 50               |
| 4     | Tes-ADH           | <5             | -                |
| 5     | ADH-T             | <5             | -                |
| 6     | ADH-A             | 5              | >99              |
| 7     | evo-1.1.200       | 6              | rac              |
| 8     | KRED-P1-A04       | 5              | 40 (S)           |
| 9     | KRED-P1-A12       | 49             | 94 (S)           |
| 10    | KRED-P1-B02       | 42             | 95 (S)           |
| 11    | KRED-P1-B05       | 14             | 96 (S)           |
| 12    | KRED-P1-B10       | 64             | 96 (S)           |
| 13    | KRED-P1-B12       | 83             | 99 (S)           |
| 14    | KRED-P1-C01       | 81             | 99 (S)           |
| 15    | KRED-P1-H08       | 86             | 99 (S)           |
| 16    | KRED-P2-B02       | 73             | 86 (S)           |
| 17    | KRED-P2-C02       | 84             | 74 (S)           |
| 18    | KRED-P2-D03       | 77             | 98 (S)           |
| 19    | KRED-P2-D11       | 23             | 97 (S)           |
| 20    | KRED-P2-D12       | 89             | 98 (S)           |
| 21    | KRED-P2-G03       | 38             | 78 (S)           |
| 22    | KRED-P2-H07       | 7              | 7 (S)            |
| 23    | KRED-P3-B03       | <5             | -                |
| 24    | KRED-P3-G09       | 7              | 76 (R)           |

* Conversion values were measured by HPLC (see the experimental section for further details and Supporting Information for the correction factor 1.48 employed after developing calibration curves).

* Enantiomeric excess values measured by HPLC.

* A complex mixture of several products was observed, including the starting material 2a and the final product 3a (n.m.: not measured).

At this point, we decided to turn our attention to commercially available ADHs, including the evo-1.1.200 from Evoxx Technologies GmbH (entry 7), which has shown to be a selective enzyme for the reduction of a wide panel of ketones [48], and a kit from Codexis Inc. including 17 ketoreductases (KREDs, entries 8-24). Gratefully, it was found that some of the tested enzymes led to conversions to (S)-3a higher than 80% with excellent selectivity (entries 13, 14, 15 and 20): KRED-P1-B12 (83% conversion, 99% ee), KRED-P1-C01 (81% conversion, 99% ee), KRED-P1-H08
(86% conversion, 99% ee), and KRED-P2-D12 (89% conversion, 98% ee). It is worth mentioning that in all cases the alcohol (S)-3a was formed as the sole product, without the presence of any detectable side product.

2.2.2. Bioreduction of α-Diazo-β-keto Esters 2b–i

After the successful development of stereoselective bioreduction processes for the keto ester 2a (summarized in Table 3, entries 1–4), the bioreduction of other α-diazo-β-keto esters 2b–i was also investigated, revealing distinct reactivity patterns as highlighted in Table 3 (complete screenings have been included in the Supplementary Material, Tables S11–S18).

Table 3. Selected examples for the stereoselective bioreduction of α-diazo-β-keto esters 2a–i.

| Entry | Substrate | R¹ | ADH/KRED | Conversion (%) | ee 3 (%) |
|-------|-----------|----|-----------|---------------|---------|
| 1     | 2a        | NCH₃| KRED-P1-B12| 83            | 99 (S)  |
| 2     | 2a        | NCH₃| KRED-P1-C01| 81            | 99 (S)  |
| 3     | 2a        | NCH₃| KRED-P1-H08| 86            | 99 (S)  |
| 4     | 2a        | NCH₃| KRED-P2-D12| 89            | 98 (S)  |
| 5     | 2b        | CIC₆H₅| KRED-P2-D11| 99            | 99 (R)  |
| 6     | 2b        | CIC₆H₅| KRED-P2-D12| >99           | 98 (R)  |
| 7     | 2b        | CIC₆H₅| KRED-P2-G03| 99            | 98 (R)  |
| 8     | 2b        | CIC₆H₅| LB-ADH     | 71            | 99 (R)  |
| 9     | 2c        | CICH₃| KRED-P1-A12| >99           | >99 (R) |
| 10    | 2c        | CICH₃| KRED-P2-D11| 99            | 96 (R)  |
| 11    | 2c        | CICH₃| KRED-P2-D12| 99            | 99 (R)  |
| 12    | 2c        | CICH₃| KRED-P2-G03| 99            | 98 (R)  |
| 13    | 2c        | CICH₃| LB-ADH     | 71            | 98 (R)  |
| 14    | 2d        | CH₃ | KRED-P1-C01| 60            | 85 (S)  |
| 15    | 2d        | CH₃ | KRED-P2-B02| 50            | 67 (S)  |
| 16    | 2e        | CH₃OCH₂| CH₃| KRED-P1-C01| 82            | 99 (R)  |
| 17    | 2e        | CH₃OCH₂| CH₃| KRED-P2-B02| 98            | 81 (R)  |
| 18    | 2f        | CH₃ | KRED-P1-B02| 93            | 99 (S)  |
| 19    | 2f        | CH₃ | KRED-P1-B05| 84            | 98 (S)  |
| 20    | 2f        | CH₃ | KRED-P2-D11| 67            | 97 (S)  |
| 21    | 2f        | CH₃ | KRED-P2-G03| 73            | 59 (R)  |
| 22    | 2g        | CH₃ | KRED-P1-C01| 40            | 96 (S)  |
| 23    | 2g        | CH₃ | KRED-P2-B02| 60            | 95 (S)  |
| 24    | 2g        | CH₃ | KRED-P2-C02| 50            | 96 (S)  |
| 25    | 2h        | BrCH₃| CH₃CH₂| KRED-P2-D11| 98            | 98 (R)  |
| 26    | 2h        | BrCH₃| CH₃CH₂| KRED-P2-D12| 99            | 91 (R)  |
| 27    | 2h        | BrCH₃| CH₃CH₂| KRED-P2-G03| 99            | 90 (R)  |
| 28    | 2i        | NCSCH₂| CH₃| KRED-P2-D11| 92            | 97 (R)  |
| 29    | 2i        | NCSCH₂| CH₃| KRED-P2-D12| 99            | 98 (R)  |
| 30    | 2i        | NCSCH₂| CH₃| KRED-P2-G03| 99            | 97 (R)  |

*a Conversion and enantiomeric excess values measured by HPLC.

Overall, the best results in terms of activity and selectivity were obtained with the commercially available KREDs. Remarkably, for the α-diazo-β-keto esters bearing different substitutions at the γ-position (Cl for 2b and 2c, Br for 2h, and SCN for 2i), it was found that three of the ketoreductases studied (KRED-P2-D11, KRED-P2-D12, and KRED-P2-G03) displayed excellent activity (92 to >99% conversion) and selectivity values (90 to >99% ee; entries 5–7, 10–12, 25–30) towards the formation of the corresponding (R)-alcohols.

In-house made ADHs did not perform the reduction of the azido-substituted keto ester 2a satisfactorily (see Section 2.2.1), but LB-ADH was able to reduce both chloro analogues 2b and 2c to the corresponding alcohols 3b and 3c with reasonable conversion (71%) and high selectivity (99 and
98% ee, respectively, entries 8 and 13). At this point, the influence of the alkoxy group of the ester functionality was also considered, and a simple comparison between ethyl 4-chloro-2-diazo-3-oxobutanoate (2b, entries 5-7) and its corresponding methyl ester 2c (entries 10-12) demonstrated that there were not significant differences among KRED-P2-D11, KRED-P2-D12, and KRED-P2-G03, with the reductions reaching quantitative conversion and excellent selectivity in all cases.

Lower conversions and selectivity were observed for the other methyl esters studied (2d and 2e), with better results being obtained through the use of either KRED-P1-C01 (for 2d: 60% conversion and 85% ee, entry 14; for 2e: 82% conversion and 99% ee), entry 16) or KRED-P2-B02 (for 2d: 50% conversion and 67% ee, entry 15; for 2e: 98% conversion and 81% ee, entry 17). Similarly, the reactivity of ethyl 2-diazo-3-oxo-3-phenylpropanoate (2f) was also dependent of the conditions studied. For instance, only a 67% conversion to the alcohol (S)-3f was reached with KRED-P2-D11 (97% ee, entry 20), although higher conversions (93 and 84%) and excellent selectivity (98 and 99% ee) were respectively found with KRED-P1-B02 (entry 18) and KRED-P1-B05 (entry 19). Interestingly, the use of KRED-P2-G03 led to the formation of the (R)-enantiomer of 3f instead of the expected (S)-configuration (entry 21), although in this case the conversion (73%) and the selectivity (59% ee) was not as high as those observed for the formation of (S)-3f. A similar trend was also noticed when the bioreduction of the benzyl ester 2g was evaluated, with KRED-P1-C01 displaying a very good selectivity (96% ee) but a low conversion (40%, entry 22), while both KRED-P2-B02 and KRED-P2-C02 led to higher conversions (60 and 50%, respectively) maintaining the stereoselection level (95 and 96% ee, entries 23 and 24).

2.2.3. Absolute Configuration Assignment for the Optically Active Hydroxy Esters 3a–i Obtained through the Bioreduction Process

The best biotransformations in terms of conversion and enantiomeric excess were successfully scaled-up to a 0.23 mmol scale, moving the reactions from Eppendorf tubes to Erlenmeyer flasks. The preparative reductions were carried out with seven (2a-c,e,f,h,i) out of the nine α-diazo-β-keto esters 2a–i that gave conversions higher than 80% in the optimization studies (see Table 3). In all cases, almost identical results in terms of activity and selectivity were observed for the production of 3a–c,e,f,h,i. Thus, the corresponding optically active α-diazo-β-hydroxy esters 3a–c,e,f,h,i were obtained at 82–99% conversion (73–96% isolated yield) and 96–99% ee after 24 h at 30 °C and 250 rpm (see the Materials and Methods section and the Supporting Information for further details). In all cases, positive values were found after measurements of the specific optical rotation values, allowing the direct comparison between the alcohol 3f obtained from the bioreduction of 2f with KRED-P1-B02 \((\alpha)_{D}^{25} = +19.3 (c 0.42, \text{CHCl}_3, 98\% \text{ ee})\) and the data reported in the literature for \((S)-3f \ (49) \ ((\alpha)_{D}^{25} = +21.9 (c 0.42, \text{CHCl}_3, 91\% \text{ ee}))\).

Additional trends in the behavior of the ADHs were observed after performing the bioreduction experiments. On the one hand, Sy-ADH and ADH-A, which are considered to act following the Prelog selectivity, displayed in all cases the opposite stereopreference to anti-Prelog enzymes such as LB-ADH and evo-1.1.200. On the other hand, the commercially available KREDs led to the same enantiomers as those observed for LB-ADH and evo-1.1.200. For these reasons, and taking into consideration the priority changes when assigning the absolute configurations, the synthesis of α-diazo-β-hydroxy esters \((S)-3a,d,f,g\) and \((R)-3b,c,e,h,i\) is here claimed (Scheme of Table 3).

3. Materials and Methods

3.1. General Methods

Alcohol dehydrogenases and glucose dehydrogenase (GDH-105) were purchased from Codexis Inc. (Redwood City, California, United States), while evo-1.1.200 ADH was acquired from Evoxx technologies GmbH (Monheim am Rhein, Germany). In-house made ADHs were overexpressed in E. coli: Rhodococcus ruber (ADH-A), Thermoanaerobacter species (ADH-T), Lactobacillus brevis
(LB-ADH), Ralstonia species (Ras-ADH), Sphingobium yanoikuyae (Sy-ADH), and Thermoanaerobacter ethanolicus (Tes-ADH) [50]. d-Glucose, NADPH, NADH, \( \beta \)-keto esters 1, and the other reagents for the development of chemoenzymatic transformations, were acquired from Sigma-Aldrich (Madrid, Spain) and used as received. \( \beta \)-Keto esters 1h and 1i were chemically synthesized by known methods, exhibiting physical and spectral data in agreement with those reported in the literature [51,52].

\( ^1H, ^13C, \) and DEPT NMR spectra were recorded on a Bruker AV300 MHz spectrometer (Bruker Co., Faellanden, Switzerland). All chemical shifts (\( \delta \)) are given in parts per million (ppm) and referenced to the residual solvent signal as the internal standard (for some experiments the carbon atom directly linked to the diazo functionality was not detected). IR spectra were recorded on a Jasco FT/IR-4700 spectrophotometer (Jasco-Spain, Madrid, Spain), and \( \nu_{\text{max}} \) values are given in cm\(^{-1}\) for the main absorption bands. Melting points were measured in a Stuart apparatus SMP3 (Bibby Sterlin, Staffordshire, UK) by introducing the samples in open capillary tubes, and the measurements are uncorrected. High-resolution mass spectra (HRMS) experiments were carried out by electrospray ionization in positive mode (ESI) using a VG AutoSpec Q high-resolution mass spectrometer (Fison Instrument, Milford, Massachusetts, USA). Measurement of the optical rotation values was carried out at 590 nm on an Autopol IV Automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA).

High-performance liquid chromatography (HPLC) analyses were performed with an Agilent 1260 Infinity chromatograph with UV detector at 210 nm (Agilent Technologies, Inc., Wilmington, DE, USA) using chiral HPLC columns (Chiral Technologies Daicel Group, Illkirch Cedex, France) such as Chiralcel OJ-H (25 cm \( \times \) 4.6 mm, 5 \( \mu \)m particle size) and Chiralad AD-H (25 cm \( \times \) 4.6 mm, 5 \( \mu \)m particle size) with an isocratic flow of 0.8 mL/min. Thin-layer chromatography (TLC) was conducted with Merck Silica Gel 60 F254 precoated plates (Merck KGaA, Darmstadt, Germany) and were visualized with UV, potassium permanganate, and vanillin stain. Column chromatography was performed using silica gel 60 (230-240 mesh, Merck KGaA, Darmstadt, Germany).

3.2. Chemical Synthesis of Ethyl 4-Azido-2-diazo-3-oxobutanoate (2a)

Azido diazo ester 2a was prepared according to the one-pot procedure from commercially available ethyl 4-chloroacetocetate (1b), as described elsewhere [37]. The product 2a was obtained as a yellowish oil (86% yield; see Table 1) and its spectral data are in agreement with those reported in the literature [37,53]; \( R_f \) (4:1 hexane/EtOAc): 0.43; IR (neat): 2145, 2105, 1715, and 1665 cm\(^{-1}\); \( ^1H \)-NMR (CDCl\(_3\), 300 MHz) \( \delta \) 4.37 (s, 2H), 4.28 (\( q, J = 7.0 \) Hz, 2H), and 1.31 ppm (\( t, J = 7.0 \) Hz, 3H); \( ^13C \)-NMR (CDCl\(_3\), 75 MHz) \( \delta \) 186.9, 161.3, 62.4, 56.4, and 14.7 ppm.

3.3. Chemical Synthesis of \( \alpha \)-Diazo-\( \beta \)-keto Esters 2b–i

The synthesis of \( \alpha \)-diazo-\( \beta \)-keto esters 2b–i was performed following a similar protocol to the one described in the literature [37]. t-BuNH\(_2\) (5.0 mmol, 525 \( \mu \)L) was added dropwise to a solution of the corresponding \( \beta \)-ketoester 1b–i (5.0 mmol) and 4-acetamidobenzensulfonyl azide (5.0 mmol, 1.20 g) in dry THF (10 mL) under inert atmosphere at 25 °C. The mixture was stirred at room temperature and monitored by TLC analysis (4:1 hexane/EtOAc). After complete consumption of the starting material, the mixture was diluted with CH\(_2\)Cl\(_2\) (20 mL), washed with brine (15 mL), dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. After complete removal of the solvent, the residue was triturated with diethyl ether (3 \( \times \) 10 mL) and the resulting mixture was again concentrated under reduced pressure. The final solid residue was repeatedly triturated with hexane (3 \( \times \) 10 mL) to separate out the insoluble ABSNH\(_2\); by decantation. The resulting mixture was filtered, concentrated under reduced pressure, and purified by column chromatography on silica gel (4:1 hexane/EtOAc), obtaining the corresponding \( \alpha \)-diazo-\( \beta \)-ketoesters 2b–i as oils (81-96% yield; see Table 1).

Ethyl 4-chloro-2-diazo-3-oxobutanoate (2b) [53]; Yellowish oil (96% yield); \( R_f \) (4:1 hexane/EtOAc): 0.48; IR (neat): 2142, 1715, and 1670 cm\(^{-1}\); \( ^1H \)-NMR (CDCl\(_3\), 300 MHz) \( \delta \) 4.60 (s, 2H), 4.30 (\( q, J = 7.1 \) Hz, 2H),
and 1.32 ppm (t, J = 7.1 Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 184.4, 161.1, 76.2, 62.3, 47.4, and 14.6 ppm.

**Methyl 4-chloro-2-diazo-3-oxobutanoate (2c)** [54]: Yellowish oil (90% yield); \(R_t\) (4:1 hexane/EtOAc): 0.30; IR (neat) 2144, 1715, and 1679 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.60 (s, 2H), and 3.85 ppm (s, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 184.3, 161.6, 76.2, 53.0, and 47.3 ppm.

**Methyl 2-diazo-3-oxobutanoate (2d)** [55]: Yellowish oil (81% yield); \(R_t\) (4:1 hexane/EtOAc): 0.41; IR (neat) 2139, 1720, and 1657 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 3.84 (s, 3H), and 2.48 ppm (s, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 190.1, 161.8, 76.2, 52.2, and 28.2 ppm.

**Methyl 2-diazo-4-methoxy-3-oxobutanoate (2e)** [56]: Yellowish oil (92% yield); \(R_t\) (3:1 hexane/EtOAc): 0.43; IR (neat) 2100, 1731, and 1684 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.51 (s, 2H), 3.82 (s, 3H), and 3.45 ppm (s, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 189.3, 162.0, 76.2, 75.2, 60.0, and 52.8 ppm.

**Ethyl 2-diazo-3-oxo-3-phenylpropanoate (2f)** [55]: Yellowish oil (95% yield); \(R_t\) (4:1 hexane/EtOAc): 0.51; IR (neat) 2184, 1726, and 1649 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.61-7.58 (m, 2H), 7.50-7.35 (m, 3H), 4.19 (q, \(J = 7.1\) Hz, 2H), and 1.20 ppm (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 187.4, 161.4, 137.5, 132.7, 128.7 (2 × CH), 128.3 (2 × CH), 76.6, 62.0, and 14.6 ppm.

**Benzyl 2-diazo-3-oxobutanoate (2g)** [55]: Yellowish oil (90% yield); \(R_t\) (4:1 hexane/EtOAc): 0.57; IR (neat) 2097, 1737, and 1682 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 7.38 (brs, 5H), 5.27 (s, 2H), and 2.48 ppm (s, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 190.0, 161.3, 135.1, 128.7 (2 × CH), 128.6, 128.4 (2 × CH), 76.4, 67.0, and 28.3 ppm.

**Ethyl 4-bromo-2-diazo-3-oxobutanoate (2h)** [53]: Yellowish oil (94% yield); \(R_t\) (4:1 hexane/EtOAc): 0.46; IR (neat) 2140, 1715, and 1655 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.39 (s, 2H), 4.31 (q, \(J = 7.0\) Hz, 2H), and 1.32 ppm (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 184.1, 160.8, 62.0, 29.6, and 14.3 ppm.

**Ethyl 2-diazo-3-oxo-4-thiocyanobutanoate (2i)** [53]: Pale orange oil (88% yield); \(R_t\) (4:1 hexane/EtOAc): 0.25; IR (neat) 2145, 1710, and 1655 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.37-4.30 (m, 4H), and 1.35 ppm (t, \(J = 6.0\) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 184.1, 161.2, 111.8, 62.8, 41.8, and 14.7 ppm.

### 3.4. Chemical Reduction of α-Diazo-β-keto Esters 2a–i using Sodium Borohydride

Sodium borohydride (1.0 mmol, 37.8 mg) was added at 0 °C to a solution of the corresponding α-diazo-β-keto ester 2a–i (1.0 mmol) in dry THF (2 mL) under an inert atmosphere. The mixture was stirred at room temperature for 30 min and monitored by TLC analysis (4:1 hexane/EtOAc). Next, the reaction was quenched with an aqueous saturated solution of NH\(_4\)Cl (1 mL); the resulting mixture was extracted with EtOAc (3 × 5 mL), and the organic phases were combined and washed with brine (1 × 15 mL). The resulting organic phase was dried over Na\(_2\)SO\(_4\), filtered, and concentrated under reduced pressure. Finally, the crude product was purified by column chromatography on silica gel (4:1 hexane/EtOAc) to give the corresponding α-diazo-β-hydroxy esters 3a–i as oils (62–85% yield).

**Ethyl 4-azido-2-diazo-3-hydroxybutanoate (3a)**: Yellowish oil (71% yield); \(R_t\) (4:1 hexane/EtOAc): 0.32; IR (neat) 3371, 2102, and 1684 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.77 (q, \(J = 6.1\) Hz, 1H), 4.25 (q, \(J = 9.0\) Hz, 2H), 3.55 (d, \(J = 6.1\) Hz, 2H), 2.87 (brs, 1H), and 1.29 ppm (t, \(J = 9.0\) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 166.1, 65.7, 61.4, 54.3, and 14.4 ppm; HRMS (ESI\(^{+}\), m/z): calcld for (C\(_6\)H\(_9\)N\(_2\)O\(_3\)+)\(^{+}\) (M + Na\(^{+}\)): 222.0599, found 222.0598.

**Ethyl 4-chloro-2-diazo-3-hydroxybutanoate (3b)**: Yellowish oil (75% yield); \(R_t\) (4:1 hexane/EtOAc): 0.36; IR (neat) 3386, 2115, and 1667 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.77 (t, \(J = 6.0\) Hz, 1H), 4.24 (q, \(J = 7.1\) Hz, 2H), 3.73 (d, \(J = 6.0\) Hz, 2H), 3.42 (brs, 1H), and 1.28 ppm (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C-NMR (CDCl\(_3\), 75 MHz) \(\delta\) 165.9, 66.8, 61.3, 46.8, and 14.4 ppm; HRMS (ESI\(^{+}\), m/z): calcld for (C\(_6\)H\(_9\)ClN\(_2\)O\(_3\)+)\(^{+}\) (M + Na\(^{+}\)): 215.0195, found 215.0194.

**Methyl 4-chloro-2-diazo-3-hydroxybutanoate (3c)**: Yellowish oil (69% yield); \(R_t\) (4:1 hexane/EtOAc): 0.19; IR (neat) 3419, 2103, and 1683 cm\(^{-1}\); \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz) \(\delta\) 4.79-4.77 (m, 1H), 3.79 (s, 3H),...
Molecules 2020, 25, 931

3.77-3.71 (m, 2H), and 3.17 ppm (brs, 1H). 13C-NMR (CDCl3, 75 MHz) δ 166.6, 67.2, 52.6, and 47.2 ppm; HRMS (ESI+, m/z): calcd for (C5H9ClN2NaO4+ (M + Na)+: 201.037, found 201.0037.

*Methyl 2-diazo-3-hydroxybutanoate (3d)*: Yellowish oil (62% yield); Rf (4:1 hexane/EtOAc): 0.31; IR (neat): 3392, 2135, and 1652 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 4.95-4.90 (m, 1H), 3.79 (s, 3H), 2.58 (brs, 1H), and 1.41 ppm (d, J = 6.5 Hz, 3H); 13C-NMR (CDCl3, 75 MHz) δ 165.4, 64.5, 59.5, 54.9, and 19.6 ppm. HRMS (ESI+, m/z): calcd for (C6H9NaN2O4+ (M + Na)+: 167.0421, found 167.0427.

*Methyl 2-diazo-3-hydroxy-4-methoxybutanoate (3e)*: Yellowish oil (78% yield); Rf (3:1 hexane/EtOAc): 0.33; IR (neat): 3418, 2100, and 1684 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 4.79 (brs, 1H), 3.79 (s, 3H), 3.69-3.54 (m, 2H), 3.42 (s, 3H), and 3.08 ppm (brs, 1H); 13C-NMR (CDCl3, 75 MHz) δ 166.4, 74.0, 65.0, 59.2, and 52.0 ppm; HRMS (ESI+, m/z): calcd for (C7H12O3NaN+ (M + Na)+: 197.0537, found 197.0533.

*Ethyl 2-diazo-3-hydroxy-3-phenylpropanoate (3f)* [49]: Yellowish oil (85% yield); Rf (4:1 hexane/EtOAc): 0.42; IR (neat): 3419, 2098, and 1665 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 7.45-7.30 (m, 5H), 5.91 (s, 1H), 4.28 (q, J = 6.1 Hz, 2H), 3.10 (brs, 1H), and 1.30 ppm (t, J = 6.1 Hz, 3H); 13C-NMR (CDCl3, 75 MHz) δ 166.4, 138.8, 128.0 (2 × CH), 128.4, 125.7 (2 × CH), 68.8, 61.2, and 14.5 ppm; HRMS (ESI+, m/z): calcd for (C7H12O3NaN+ (M + Na)+: 243.0743, found 243.0740.

*Benzyl 2-diazo-3-hydroxybutanoate (3g)*: Yellowish oil (78% yield); Rf (4:1 hexane/EtOAc): 0.44; IR (neat): 3397, 2097, and 1688 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 7.37-7.34 (m, 5H), 5.22 (s, 2H), 4.94 (q, J = 6.6 Hz, 1H), 2.84 (brs, 2H), and 1.40 ppm (d, J = 6.6 Hz, 3H); 13C-NMR (CDCl3, 75 MHz) δ 165.5, 136.1, 129.0 (2 × CH), 128.8, 128.6 (2 × CH), 68.1, 62.0, and 21.0 ppm; HRMS (ESI+, m/z): calcd for (C8H14N2O3+ (M + Na)+: 243.0740, found 243.0740.

*Ethyl 4-bromo-2-diazo-3-hydroxybutanoate (3h)*: Yellowish oil (71% yield); Rf (4:1 hexane/EtOAc): 0.34; IR (neat): 3402, 2079, and 1695 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 4.78 (brs, 1H), 4.25 (q, J = 7.1 Hz, 2H), 3.76-3.73 (m, 2H), 3.18 (brs, 1H), and 1.29 ppm (t, J = 7.1 Hz, 3H); 13C-NMR (CDCl3, 75 MHz) δ 165.7, 66.6, 61.2, 35.6, and 14.4 ppm; HRMS (ESI+, m/z): calcd for (C9H16BrN2O3+ (M + Na)+: 258.9698, found 258.9689.

*Ethyl 2-diazo-3-hydroxy-4-thiocyanobutanoate (3i)*: Yellowish oil (77% yield); Rf (4:1 hexane/EtOAc): 0.17; IR (neat): 3381, 2138, and 1653 cm⁻¹; 1H-NMR (CDCl3, 300 MHz) δ 4.87 (q, J = 9.1 Hz, 1H), 3.42 (brs, 2H), 3.34-3.30 (m, 2H), and 1.29 ppm (t, J = 9.1 Hz, 3H); 13C-NMR (CDCl3, 75 MHz) δ 165.6, 111.7, 66.5, 61.5, 38.4, and 14.4 ppm; HRMS (ESI+, m/z): calcd for (C7H8N2O3S+ (M + Na)+: 215.0211, found 215.0365.

3.5. Bioreduction of α-Diazo-β-keto Esters 2a−i using Sy-ADH, TES-ADH, and ADH-T

The selected ADH (12 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a−i (0.015 mmol), DMSO (56 μL), PrOH (30 μL), a 10 mM aqueous solution of NADPH (60 μL), and a 50 mM Tris/HCl pH 7.5 buffer (454 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a−i by HPLC.

3.6. Bioreduction of α-Diazo-β-keto Esters 2a−i using LB-ADH

LB-ADH (12 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a−i (0.015 mmol), DMSO (56 μL), PrOH (30 μL), a 10 mM aqueous solution of MgCl2 (60 μL), a 10 mM aqueous solution of NADPH (60 μL), and a 50 mM Tris/HCl pH 7.5 buffer (394 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling
nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–i by HPLC.

3.7. Bioreduction of α-Diazo-β-keto Esters 2a–i using ADH-A

ADH-A (12 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a–i (0.015 mmol), DMSO (56 μL), PrOH (30 μL), a 10 mM aqueous solution of NADH (60 μL), and a 50 mM Tris/HCl pH 7.5 buffer (454 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–i by HPLC.

3.8. Bioreduction of α-Diazo-β-keto Esters 2a–i using Ras-ADH

Ras-ADH (12 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a–i (0.015 mmol), DMSO (75 μL), an aqueous solution of GDH (60 μL, 10 U), a 50 mM aqueous solution of α-glucose (60 μL), a 10 mM aqueous solution of NADPH (60 μL), and a 50 mM Tris/HCl pH 7.5 buffer (345 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–i by HPLC.

3.9. Bioreduction of α-Diazo-β-keto Esters 2a–i using evo-1.1.200

Evo-1.1.200 (12 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a–i (0.015 mmol), DMSO (56 μL), PrOH (25 μL), a 10 mM aqueous solution of MgCl2 (50 μL), a 10 mM aqueous solution of NADH (50 μL), and a 50 mM Tris/HCl pH 7.5 buffer (319 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–i by HPLC.

3.10. Bioreduction of α-Diazo-β-keto Esters 2a–i using Commercially Available ADHs from Codexis Inc.

The selected commercially available Codexis KRED (1 mg) was added to a 1.5 mL Eppendorf tube containing the corresponding α-diazo-β-ketoester 2a–i (0.013 mmol), PrOH (95 μL), and an aqueous solution with Na3PO4 (128 mM), MgSO4 (1.7 mM) and NADP+ (1.1 mM) resulting in pH 7.0 (450 μL). Then, the Eppendorf tube was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 0.5 mL) and the combined organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it, and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–i by HPLC.

3.11. Semi-preparative Bioreduction of α-Diazo-β-keto esters 2a–c,e,f,h,i using Commercially Available ADHs from Codexis Inc.

The selected commercially available Codexis KRED (12 mg) was added to a 25 mL Erlenmeyer flask containing the corresponding α-diazo-β-ketoester 2a–c,e,f,h,i (0.23 mmol), PrOH (920 μL), and an aqueous solution with Na3PO4 (128 mM), MgSO4 (1.7 mM), and NADP+ (1.1 mM) resulting in pH 7.0 (8.28 mL). Then, the Erlenmeyer flask was closed and kept under orbital shaking at 250 rpm at 30 °C for 24 h. After this time, the product was extracted with EtOAc (3 × 10 mL), and the combined
organic layers were dried over anhydrous Na2SO4. Finally, the solvent was carefully evaporated by bubbling nitrogen gas through it and an aliquot was taken for the measurement of conversion and enantiomeric excess values of the corresponding α-diazo-β-hydroxy ester 3a–c,e,f,h,i by HPLC. At this point, the resulting reaction crude was purified by column chromatography on silica gel (4:1 hexane:EtOAc) to give the pure corresponding α-diazo-β-hydroxy esters 3a–c,e,f,h,i as yellowish oils.

(S)-3a: [α]D = +5.4 (c 0.1, CHCl3, 97% ee) after bioreduction with KRED-P2-D12 (83% conversion, 74% isolated yield).

(R)-3b: [α]D = +7.5 (c 0.1, CHCl3, 96% ee) after bioreduction with KRED-P2-D12 (99% conversion, 86% isolated yield).

(R)-3c: [α]D = +7.1 (c 0.1, CHCl3, 98% ee) after bioreduction with KRED-P2-D12 (99% conversion, 96% isolated yield).

(R)-3e: [α]D = +9.4 (c 0.1, CHCl3, 99% ee) after bioreduction with KRED-P1-C01 (82% conversion, 73% isolated yield).

(S)-3f: [α]D = +19.3 (c 0.42, CHCl3, 98% ee) after bioreduction with KRED-P1-B02 (91% conversion, 83% isolated yield).

(R)-3h: [α]D = +8.5 (c 0.1, CHCl3, 96% ee) after bioreduction with KRED-P2-D11 (97% conversion, 89% isolated yield).

(R)-3i: [α]D = +7.8 (c 0.1, CHCl3, 96% ee) after bioreduction with KRED-P2-D12 (97% conversion, 91% isolated yield).

4. Conclusions

A family of α-diazo-β-keto esters 2a–i were chemically synthesized in good to excellent yields (81–96%), which were later successfully employed as substrates for bioreduction using a variety of E. coli overexpressed and commercially available alcohol dehydrogenases to give the corresponding optically active α-diazo-β-hydroxy esters 3a–i. Different structural patterns were considered, including the substitution at the adjacent position of the ketone group and in the alkoxy portion of the ester functionality. For most substrates, in particular, those bearing heteroatoms substituted at the 4-position (Cl, Br or SCN), the commercial enzymes KRED-P2-D11, KRED-P2-D12, and/or KRED-P2-G03 led to complete conversions to the corresponding alcohols 3 with excellent selectivities (97–99% ee). Alternatively, KRED-P1-C01 and KRED-P2-B02 were shown to be effective for the less reactive substrates, while the conversions decreased significantly when considering methyl ketones (up to 60% conversion). On the other hand, the type of the alkyl ester studied was not as relevant for the bioreduction, with methyl, ethyl, or benzyl esters being appropriate substrates to deliver the corresponding alcohols with high selectivity (85 to >99% ee) depending on the choice of the enzyme. Thus, straightforward access to the corresponding optically active α-diazo-β-hydroxy esters 3a–i has been provided. They were readily obtained in a preparative scale with high conversions and excellent stereoselectivities, depending on the enzyme selection.

Supplementary Materials: A pdf file containing the structures of α-diazo-β-keto esters 2a–i and hydroxy esters 3a–i, the development of analytical methods in HPLC for the measurement of conversion and enantiomeric excess values of the bioreduction processes, extensive enzyme-catalysed screenings, and full characterization of novel compounds by NMR spectra (1H and 13C-NMR experiments) is available on-line. Figure S1. Structures of α-diazo-β-keto esters 2a–i and the corresponding hydroxy esters 3a–i described in this contribution; Table S1. Retention times of α-diazo-β-keto esters 2a–i and their corresponding alcohols 3a–i in HPLC analyses; Tables S2–S10. Calibrate curves in HPLC for the bioreduction of compounds 2a–i; Tables S11–S18. Extensive enzyme screenings for the bioreduction of compounds 2b–i.

Author Contributions: Conceptualization, M.M.S. and V.G.-F.; investigation, S.G.-G. and T.A.C.; writing: original draft preparation, M.M.S. and V.G.-F.; writing: review and editing, S.G.-G., T.A.C., M.M.S., and V.G.-F.; supervision, M.M.S. and V.G.-F.; funding acquisition, V.G.-F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spanish Ministry of Economy and Competitiveness (MEC) Project CTQ2016-75752-R and the Asturian Regional Government (FC-GRUPIN-IDI/2018/000181).
Acknowledgments: Authors would like to thank Wolfgang Kroutil (University of Graz, Austria) for providing us with alcohol dehydrogenases overexpressed in E. coli cells and the technical support of the Scientific-Technical Services of the University of Oviedo. M.M.S. and T.A.C. are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil) for fellowships.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Maas, G. New Syntheses of Diazocompounds. Angew. Chem. Int. Ed. 2009, 48, 8186–8195.
2. Ford, A.; Miel, H.; Ring, A.; Slattery, C.N.; Maguire, A.R.; McKervey, M.A. Modern Organic Synthesis with α-Diazoacetylcarboxyl Compounds. Chem. Rev. 2015, 115, 9981–10080.
3. Li, Y.-P.; Li, Z.-Q.; Zhu, S.-F. Recent advances in transition-metal-catalyzed asymmetric reactions of diazo compounds with electron-rich (hetero) arenes. Tetrahedron Lett. 2018, 59, 2307–2318.
4. Xiang, Y.; Wang, C.; Ding, Q.; Peng, Y. Diazocompounds: Versatile Synthons for the Synthesis of Nitrogen Heterocycles via Transition Metal-Catalyzed Cascade C-H Activation/Carbene Insertion/Annulation Reactions. Adv. Synth. Catal. 2019, 361, 919–944.
5. Empel, C.; Koenigs, R.M. Sustainable Carbene Transfer Reactions with Iron and Light. Synlett 2019, 30, 1929–1934.
6. Dutta, N.B.; Sharma, S.; Chetry, R.L.; Baishya, G. A Green Protocol for the Synthesis of α-Diazo-β-hydroxy esters and One-Pot Conversion to β-Keto-Esters and Imidazo[1,2-a]pyridine-3-carboxylates. ChemistrySelect 2019, 4, 5817–5822.
7. Chen, D.F.; Wu, P.-Y.; Gong, L.-Z. Rhodium/Chiral Urea Relay Catalysis Enables an Enantioselective Semipinacol Rearrangement/Michael Addition Cascade. Org. Lett. 2013, 15, 3958–3961.
8. Bayir, A.; Draghi, C.; Brewer. M. Preparation of Tethered Aldehyde Ynoates and Ynone by Ring Fragmentation of Cyclic γ-Oxy-β-hydroxy-α-diazo Carboxyls. J. Org. Chem. 2010, 75, 296–302.
9. Zhang, Y.; Wang, J. Recent development of reactions with α-diazoacarbonyl compounds as nucleophiles. Chem. Commun. 2009, 36, 5350–5361.
10. Xiao, F.; Liu, Y.; Wang, J. DBU-catalyzed condensation of acyldiazomethanes to aldehydes in water and a new approach to ethyl β-hydroxy α-arylacrylates. Tetrahedron Lett. 2007, 48, 1147–1149.
11. Zhdanova, O.V.; Korneev, S.M.; Nikolaev, V.A. Chemistry of Diazocarbonyl Compounds: XVIII. Synthesis and Spectral Parameters of 1,3-Dialkyl-3-hydroxy-2-diazoketones. Russ. J. Org. Chem. 2004, 40, 316–328.
12. Moody, C.J.; Morfit, C.N. Zinc Derivative of Ethyl Diazooacetate in the Synthesis of α-Diazo-β-hydroxy Esters. Synthesis 1998, 1039–1042.
13. Jiang, N.; Wang, J. DBU-promoted condensation of acyldiazomethanes to aldehydes and imines under catalytic conditions. Tetrahedron Lett. 2002, 43, 1285–1287.
14. Cuevas-Yañez, E.; Muchowski, J.M.; Cruz-Almanza, R. Metalation of α-diazocarbonyl compounds using Grignard reagents. A convenient synthesis of α-diazo-β-ketoesters and mixed esters of α-diazomalonate. Tetrahedron Lett. 2004, 45, 2417–2419.
15. Arai, S.; Hasegawa, K.; Nishida, A. One-pot synthesis of α-diazo-β-hydroxyesters under phase-transfer catalysis and application to the catalytic asymmetric aldol reaction. Tetrahedron Lett. 2004, 45, 1023–1026.
16. Li, P.; Majireck, M.M.; Korboukh, I.; Weinreb, S.M. A mild, efficient method for the oxidation of α-diazo-β-hydroxy esters to α-diazo-β-ketoesters. Tetrahedron Lett. 2008, 49, 3162–3164.
17. Hasegawa, K.; Arai, S.; Nishida, A. Synthesis of α-diazo-β-hydroxyesters through a one-pot protocol by phase-transfer catalysis: Application to enantioselective aldol-type reaction and diastereoselective synthesis of α-amino-β-hydroxyester derivatives. Tetrahedron 2006, 62, 1390–1401.
18. Yao, W.; Wang, J. Direct catalytic asymmetric aldol-type reaction of aldehydes with ethyl diazoacetate. Org. Lett. 2003, 5, 1527–1530.
19. Trost, B.M.; Malhotra, S.; Fried, B.A. Magnesium-catalyzed asymmetric direct aldol addition of ethyl diazoacetate to aromatic, aliphatic, and α,β-unsaturated aldehydes. J. Am. Chem. Soc. 2009, 131, 1674–1675.
20. Trost, B.M.; Malhotra, S.; Koscher, P.; Ellerbrock, P. The development of the enantioselective addition of ethyl diazoacetate to aldehydes: Asymmetric synthesis of 1,2-diols. *J. Am. Chem. Soc.* 2012, 134, 2075–2084.

21. Pagar, V.V.; Jadhav, A.M.; Liu, R-S. Gold-Catalyzed Formal [3 + 3] and [4 + 2] Cycloaddition Reactions of Nitrosobenzenes with Alkenylgold Carbenoids. *J. Am. Chem. Soc.* 2011, 133, 20728–20731.

22. Davies, H.M.L.; Huby, N.J.S. Enantioselective synthesis of tropanes by reaction of rhodium-stabilized vinylcarbenoids with pyrroles. *Tetrahedron Lett.* 1992, 33, 6935–6938.

23. Applegate, G.A.; Borkowtz, D.B. Exploiting Enzymatic Dynamic Reductive Kinetic Resolution (DYKKR) in Stereorestricted Synthesis. *Adv. Synth. Catal.* 2015, 357, 1619–1632.

24. Hollmann, F.; Arends, I.W.C.E.; Holtmann, D. Enzymatic reductions for the chemist. *Green Chem.* 2011, 13, 2285–2313.

25. Kallergi, M.; Kalaitzakis, D.; Smonou, I. Enzymatic Total Synthesis of Banana Volatile (S)-2-Pentyl (R)-3-Hydroxyhexanoate. *Eur. J. Org. Chem.* 2011, 3946–3950.

26. Cuetos, A.; Ríos-Martínez, A.; Bisogno, F.R.; Grischek, B.; Lavandera, I.; de Gonzalez, G.; Kroutil, W.; Gotor, V. Access to Enantiopure α-Alkyl-β-hydroxy Esters through Dynamic Kinetic Resolutions Employing Purified/Overexpressed Alcohol Dehydrogenases. *Adv. Synth. Catal.* 2012, 354, 1743–1749.

27. Zadlo, A.; Schrittweiser, J.H.; Koszelewski, D.; Kroutil, W.; Ossaszewski, R. Enantioselective Reduction of Ethyl 3-Oxo-5-phenylpentanoate with Whole-Cell Biocatalysts. *Eur. J. Org. Chem.* 2016, 1007–1011.

28. Barik, R.; Halder, J.; Nanda, S. Biocatalytic dynamic kinetic reductive resolution with ketoreductase from Klebsiella pneumoniae: The asymmetric synthesis of functionalized tetrahydropyrans. *Org. Biomol. Chem.* 2019, 17, 8571–8588.

29. Sheldon, R.A.; Brady, D. The Limits to Biocatalysis: Pushing the Envelope. *Chem. Commun.* 2018, 54, 6088–6104.

30. Sheldon, R.A.; Pereira, P.C. Biocatalysis engineering: The big picture. *Chem. Soc. Rev.* 2017, 46, 2678–2691.

31. Ferreira-Leitão, V.S.; Cammarota, M.C.; Agueiras, E.C.G.; Sá, L.R.V.; Fernandez-Lafuente, R.; Freire, D.M.G. The Protagonism of Biocatalysis in Green Chemistry and Its Environmental Benefits. *Catalysts* 2017, 7, 9.

32. Wöhlgemuth, R. Biocatalysis—Key to sustainable industrial chemistry. *Curr. Opin. Biotechnol.* 2010, 21, 713–724.

33. Coelho, P.S.; Brustad, E.M.; Kannan, A.; Arnold, F.H. Olefin Cyclopropanation via Carbene Transfer Catalyzed by Engineered Cytochrome P450 Enzymes. *Science* 2013, 339, 307–310.

34. Renata, H.; Lewis, R.D.; Sweredoski, M.J.; Moradian, A.; Hess, S.; Wang, Z.J.; Arnold, F.H. Identification of Mechanism-Based Inactivation in P450-Catalyzed Cyclopropanation Facilitates Engineering of Improved Enzymes. *J. Am. Chem. Soc.* 2016, 138, 12527–12533.

35. Waldman, A.J.; Balskus, E.P. Discovery of a Diazo-Forming Enzyme in Cremeomycin Biosynthesis. *J. Org. Chem.* 2018, 83, 7539–7546.

36. Bräse & cols recently described the chemo- and stereoselective chemoenzymatic reduction of α-diazo esters containing remote keto groups to the corresponding α-hydroxy diazo esters en route to an asymmetric synthesis of oxygenated heterocycles: Mittmann, E.; Hu, Y.; Peschke, T.; Rabe, K.S.; Niemeyer, C.M.; Bräse, S. Chemoenzymatic Synthesis of O-Containing Heterocycles from α-Diazo Esters. *ChemCatChem* 2019, 11, 5519–5523.

37. Costin, T.A.; Dutra, L.G.; Bortoluzzi, A.J.; Sá, M.M. Amine-mediated synthesis of amides from 1,3-dicarbonyl compounds through a domino diazo transfer/aminolysis process. *Tetrahedron* 2017, 73, 4549–4559.

38. Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. Biocatalytic asymmetric hydrogen transfer. *Angew. Chem. Int. Ed.* 2002, 41, 1014–1017.

39. Peters, J.; Minuth, T.; Kula, M.-R. A novel NADH-dependent carbonyl reductase with an extremely broad substrate range from Candida parapsilosis: Purification and characterization. *Enzyme Microb. Technol.* 1993, 11, 950–958.

40. Wollberg, M.; Hummel, W.; Wandrey, C.; Müller, M. Highly Regio- and Enantioselective Reduction of 3,5-Dioxocarboxylates. *Angew. Chem. Int. Ed.* 2000, 39, 4306–4308.
41. Leuchs, S.; Greiner, L. Alcohol Dehydrogenase from Lactobacillus brevis: A Versatile Robust Catalyst for Enantioselective Transformations. Chem. Biochem. Eng. Q. 2011, 2, 267–281.

42. Lavandera, I.; Kern, A.; Ferreira-Silva, B.; Glieder, A.; de Wildeman, S.; Kroutil, W. Stereoselective Bioreduction of Bulky-Bulky Ketones by a Novel ADH from Ralstonia sp. J. Org. Chem. 2008, 15, 6003–6005.

43. Man, H.; Kędziora, K.; Kulig, J.; Frank, A.; Lavandera, I.; Gotor-Fernández, V.; Rötter, D.; Hart, S.; Turkenburg, J.P.; Grogan, G. Structures of Alcohol Dehydrogenases from Ralstonia and Sphingobium spp. Reveal the Molecular Basis for Their Recognition of ‘Bulky-Bulky’ Ketones. Top. Catal. 2014, 5, 356–365.

44. Lavandera, I.; Oberdofer, G.; Gross, J.; de Wildeman, S.; Kroutil, W. Stereocomplementary Asymmetric Reduction of Bulky–Bulky Ketones by Biocatalytic Hydrogen Transfer. Eur. J. Org. Chem. 2008, 2539–2543.

45. Lavandera, I.; Kern, A.; Resch, V.; Ferreira-Silva, B.; Glieder, A.; Fabian, W.M.F.; de Wildeman, S.; Kroutil, W. One-Way Biohydrogen Transfer for Oxidation of sec-Alcohols. Org. Lett. 2008, 11, 2155–2158.

46. Heiss, C.; Laivenieks, M.; Zeikus, J.G.; Phillips. R.S. Mutation of cysteine-295 to alanine in secondary alcohol dehydrogenase from Thermoanaerobacter ethanolicus affects the enantioselectivity and substrate specificity of ketone reductions. Biorg. Med. Chem. 2001, 7, 1659–1666.

47. The formation of side products for the particular case of Ras-ADH-catalysed reaction could possibly be related to the use of the glucose dehydrogenase enzyme-coupled system as recycling cofactor purposes, so in the absence of isopropanol (tPrOH) other redox activities can be displayed by the E. coli overexpressed system, favouring the occurrence of undesired side reactions. For instance, the formation of pyrazines from ketoxime derivatives using ene-reductases has been recently reported in a patent (Kroutil, W.; Velikonge, S. Method for the preparation of substituted pyrazines via enzymatic catalysis. EP 2019 3569594 A1).

48. Könst, P.; Merkens, H.; Kara, S.; Kochius, S.; Vogel, A.; Zuhse, R.; Holtmann, D.; Arends, I.W.C.E.; Hollmann, F. Enantioselective oxidation of aldehydes catalyzed by alcohol dehydrogenase. Angew. Chem. Int. Ed. 2012, 51, 9914–9917.

49. Wang, W.; Shen, K.; Hu, X.; Wang, J.; Liu, X.; Feng, X. Highly Enantioselective Synthesis of α-Diazo-β-hydroxy Esters Using a Bifunctional Titanium Complex. Synlett 2009, 1655–1658.

50. Méndez-Sánchez, D.; Mourell-Insua, A.; Gotor-Fernández, V.; Lavandera, I. Synthesis of α-Alkyl-β-Hydroxy Amides through Biocatalytic Dynamic Kinetic Resolution Employing Alcohol Dehydrogenases. Adv. Synth. Catal. 2019, 361, 2706–2712.

51. Kato, T.; Sato, M.; Kimura, H. Studies on Keten and Its Derivatives. Part 89. Ethyl 4-Substituted Acetoacetates: Synthesis and Reaction with Diketen. J. Chem. Soc. Perkin Trans 1, 1979, 529-532.

52. Chandrushaker, V.; Ptaszek, M.; Taniguchi, M.; Lindsey, J.S. Synthesis of diverse acyclic precursors to pyrroles for studies of prebiotic routes to tetrapyrrole macrocycles. New J. Chem. 2016, 40, 8786–8808.

53. Padwa, A.; Sä, M.M.; Weingarten, M.D. Metal substituted diazo esters as substrates for cross coupling reaction. Tetrahedron 1997, 53, 2371–2386.

54. Bagley, M.C.; Buck, R.T.; Hind, S.L.; Moody, C.J. Synthesis of functionalised oxazoles and bis-oxazoles. J. Chem. Soc., Perkin Trans. 1. 1998, 591–600.

55. Dar’in, D.; Kantin, G.; Krasavin, M. A ‘sulfonyl-azide-free’ (SAFE) aqueous-phase diazo transfer reaction for parallel and diversity-oriented synthesis. Chem. Commun. 2019, 55, 5239–5242.

56. Chen, X.; Xie, Y.; Xiao, X.; Li, G.; Deng, Y.; Jiang, H.; Zeng, W. Rh(III)-catalyzed chelation-assisted intermolecular carbenoid functionalization of α-imino Csp3–H bonds. Chem. Commun. 2015, 51, 15328–15331.

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).