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"Cap-Tag"—Novel Methods for the Rapid Purification of Oligosaccharides Prepared by Automated Solid-Phase Synthesis**

Emma R. Palmacci, Michael C. Hewitt, and Peter H. Seeberger*

The automated solid-phase synthesis of oligopeptides[1] and oligonucleotides[2] is now routine and has made a major impact on the biochemistry and biotechnology of these biopolymers. Attempts to automate the synthesis of carbohydrates, the most complex class of biopolymers, on solid phase[3] and by computer-assisted planning of solution syntheses[4] have only recently been disclosed. While automated solid-phase synthesis greatly simplifies the assembly process by eliminating the time-consuming purification of intermediates, it is this very feature that often makes the final purification challenging. The removal of sequences deficient by just one unit ($n-1$ products) stemming from incomplete couplings at any stage of the synthesis can be very difficult.

Automated solid-phase synthetic protocols for peptides[5] and nucleic acids[2] often incorporate a capping step in the synthetic cycle to minimize the accumulation of $n-1$ products. Further improvements of this technique are based on the introduction of a capping agent that functions as a unique handle to aid in the purification of the final product.[6]

With the advent of combinatorial chemistry and parallel synthesis, several methods for the expedient purification of a variety of compounds have been reported. Scavenging resins allow byproducts or excess reagents to be removed by reaction of the unwanted moieties with a support-bound functional group, followed by filtration.[7] Affinity labels such as biotin or oligohistidine exploit noncovalent binding and have been extensively used for the rapid purification of tagged biomolecules[8] and synthetic compounds[9] by affinity chromatography. Most recently, the unique behavior of highly fluorinated molecules has been utilized to separate molecules containing a fluorous tag from nonfluorinated compounds by extraction with fluorous solvents or by chromatography on fluorous silica gel.[10]

Here we describe two novel capping-and-tagging (cap-tag) methods to aid in the purification of oligosaccharides

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[**] Financial support from the donors of the Petroleum Research Fund, administered by the ACS (ACS-PRF 34649-G1), Merck (Predoctoral Fellowship for E.R.P.), Boehringer-Ingelheim (Predoctoral Fellowship for E.R.P.), and the NIH (Biotechnology Training Grant for M.C.H.) is gratefully acknowledged. Funding for the MIT-DCIF Inova 501 was provided by the NSF (Award CHE-9808061). Funding for the MIT-DCIF Avance (DPX) 400 was provided by the NIH (Award 1S10RR13886-01). We thank Silicycle (Quebec City, Canada) for the generous gift of fluorous silica gel and isocyanate silica gel scavenger resin. We thank Dr. O. Plante for helpful discussions and help with the automated oligosaccharide synthesizer.

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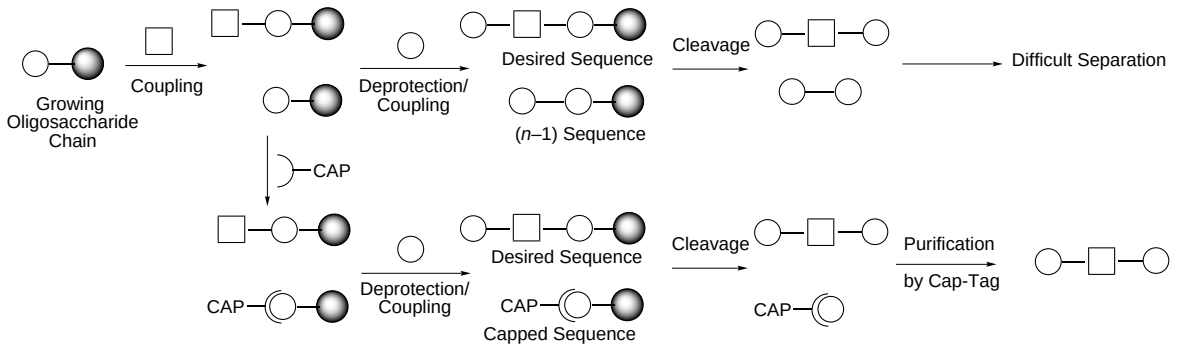
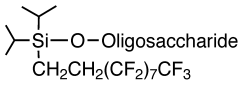


Scheme 1. Schematic overview of the cap-tag approach. Shaded circles = polymer support.

assembled by automated solid-phase synthesis. Following each coupling maneuver unconsumed hydroxyl groups that may give rise to deletion sequences are treated with a capping reagent that renders them silent in subsequent couplings (Scheme 1). The cap-tags also function as a handle that facilitates the separation of all unwanted, capped and tagged, sequences from the desired, untagged products. Two different flavors of cap-tags are introduced: an α-azidoisobutyric ester cap (A-Tag) that can be removed with an isocyanate-functionalized scavenger resin after reduction to the corresponding amine, and a fluorous silyl ether cap (F-Tag) that allows for the removal of tagged sequences by filtration through fluorous silica gel. The introduction of these cap-tags into the automated solid-phase synthesis of oligosaccharides greatly simplifies post-synthetic workup and purification, as demonstrated for three trisaccharide syntheses.

A-Tag

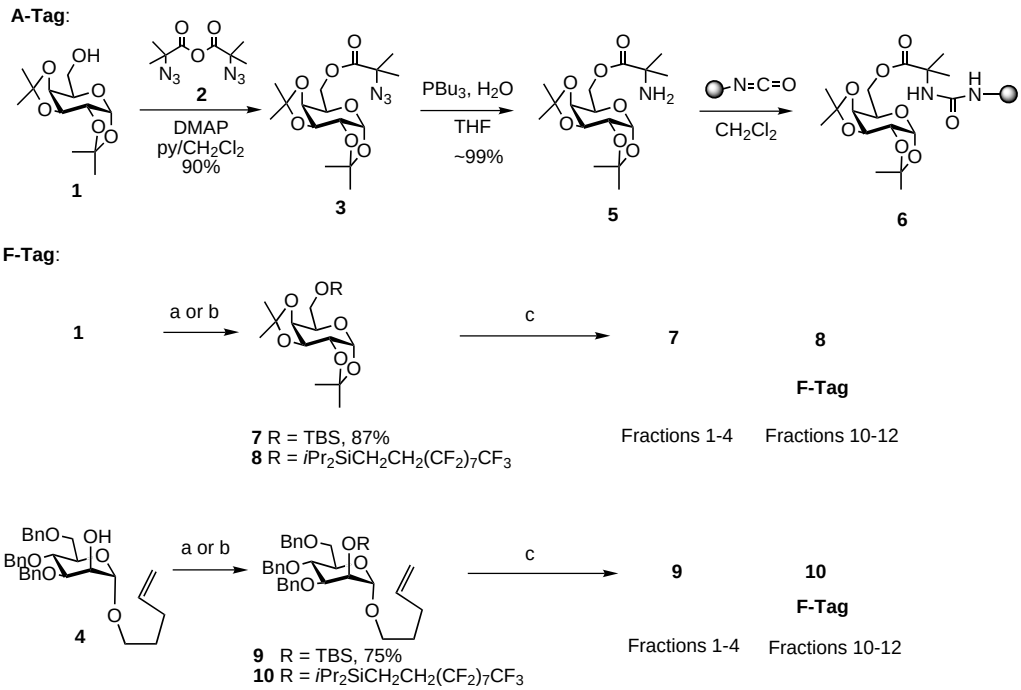


F-Tag

Several issues of critical importance must be considered in the design of cap-tags: 1) the caps must react rapidly and efficiently with hydroxyl groups that were not glycosylated in the previous coupling step; 2) the linkage formed between the hydroxyl group and the cap must be stable during subsequent coupling cycles; 3) cap-tags must be orthogonal to the protecting groups used in the synthesis, and hence different tags may be needed; 4) the cap must contain a unique handle that allows facile separation from full-length products.

Mindful of these design features, we envisioned using the A-Tag as an analogue of the robust pivaloyl ester. Synthesis of the A-Tag capping reagent **2** was straightforward and can be performed on a multigram scale. 2-Azido-2-methylpropionic acid was prepared from commercially available 2-bromo-2-methylpropionic acid,[11] and treatment with dicyclohexylcarbodiimide (DCC) furnished anhydride **2**. Alternatively, the corresponding acid chloride can be prepared and used in place of **2** to introduce the A-Tag.

Initially, we focused on model studies on monosaccharides to examine the properties of the A-Tag (Scheme 2, top). Capping of monosaccharides containing primary (**1**) and secondary (**4**) hydroxyl groups by reaction with **2** in the presence of DMAP afforded esters such as **3** in high yield. With capped monosaccharide **3** in hand, we first determined the stability of the A-Tag to different reaction conditions employed during oligosaccharide synthesis. Monosaccharides containing common temporary protecting groups were com-



Scheme 2. Model monosaccharide synthesis and scavenging. a) 4 equiv (heptadecafluorodecyl)diisopropylsilyl triflate (**11**), 6 equiv 2,6-lutidine in CH_2Cl_2 at RT; b) 1.1 equiv TBSOTf, 2 equiv 2,6-lutidine in CH_2Cl_2 at RT; c) fluorous silica gel ($Si(CH_2CH_2C_6F_{13})_3$), gradient of 80/20% H_2O to 100% MeOH. Bn = benzyl, DMAP = 4-dimethylaminopyridine, py = pyridine; TBS = tBuMe$_2$Si.

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bined with **3**, and the protective groups were removed. We found that standard levulinate deprotection conditions (hydrazine in pyridine/AcOH) did not result in removal of the A-Tag. Removal of acetate esters by treatment with sodium methoxide resulted in A-Tag cleavage and thus rendered these groups incompatible.

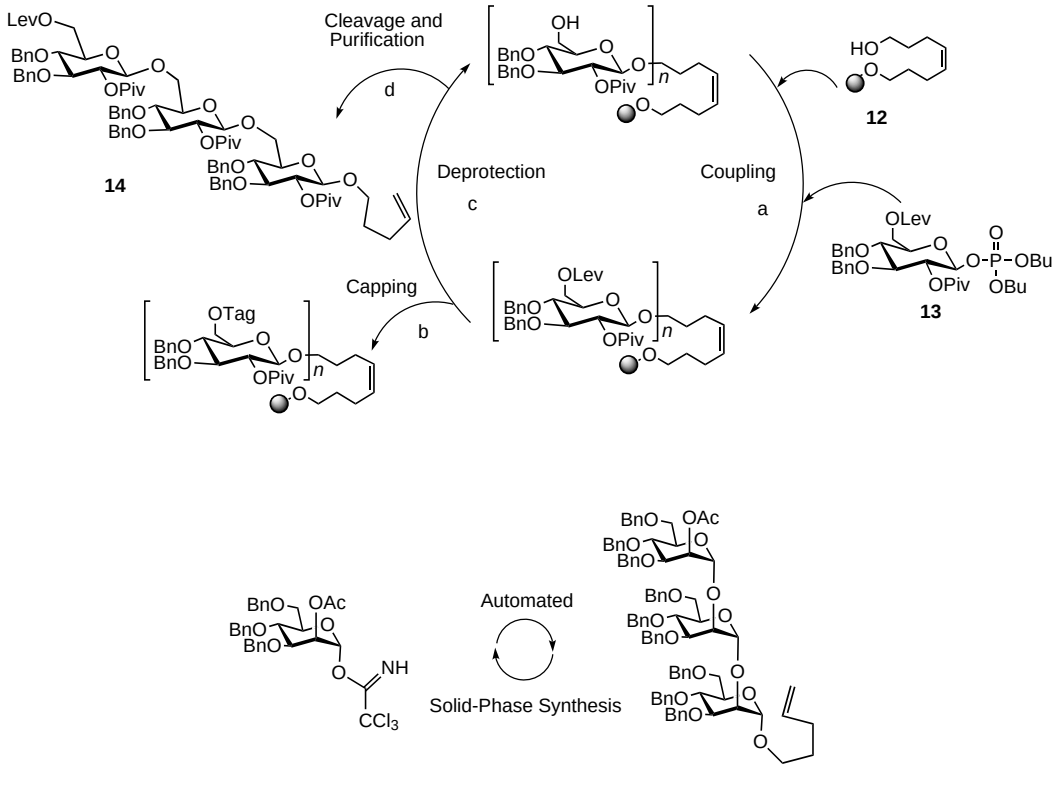
The possibility of removing molecules containing the A-Tag from reactions was also demonstrated on a monosaccharide model (Scheme 2, top). Conversion of azide **3** to amine **5** by treatment with tributylphosphane in water/THF proceeded quickly (< 5 min). Three hours after addition of an isocyanate silica gel scavenging resin to a solution of **5** in CH_2Cl_2, complete consumption of **5** was indicated by failure to detect any amine in a ninhydrin test.

Our second cap-tag incorporates a fluorinated silyl ether tag. Highly fluorinated molecules have been shown to possess unique properties that can be exploited for separating them from very similar fluorine-free compounds.[10] To explore the F-Tag concept on monosaccharide models, (heptadecafluorodecyl)diisopropylsilyl triflate (**11**)[12] was reacted with carbohydrates containing primary (**1**) and secondary (**4**) hydroxyl groups to furnish F-tagged **8** and **10** (Scheme 2, bottom). F-Tag-containing monosaccharides **8** and **10** were completely stable to treatment with sodium methoxide, even after prolonged exposure (24 h).

Silylated monosaccharides **7** and **9** were prepared to demonstrate the separation principle in the cap-tag approach. Equimolar **7**/**8** and **9**/**10** mixtures were applied to columns of tridecafluoro(Si($CH_2CH_2C_6F_{13}$)$_3$)-functionalized silica gel packed in methanol/water (4/1). Unfluorinated monosaccharides **7** and **9** were eluted immediately (fractions 1 – 4), while fluorinated compounds **8** and **10** were eluted in late fractions, only after washing the columns with 100 % methanol.

After establishing that carbohydrates could be tagged and purified with the A-Tag and the F-Tag, we investigated the effectiveness of the cap-tag strategy in the automated solid-phase synthesis of trisaccharides **14** and **16** (Scheme 3). Currently, high yields (ca. 95 % per step) in automated solid-phase oligosaccharide synthesis are ensured by addition of several equivalents of glycosylating agent and repetition of this process (double coupling).[3] To clearly demonstrate the effect of the cap-tags, only single couplings were performed in each cycle, and coupling efficiencies in the range of 85 – 90 % were expected. Syntheses of each oligosaccharide with and without the capping step but with otherwise identical coupling cycles were compared. Resin-bound oligosaccharides prepared with the help of the A-Tag were reduced with tributylphosphane before being cleaved from the solid support. After cleavage, the crude products were treated with isocyanate silica gel scavenger resin. Oligosaccharides prepared without capping were directly cleaved and were not treated with a scavenger resin.

The HPLC traces of the capped and scavenged reaction products (Figure 1) were compared to those of the reaction



Scheme 3. Top: Synthesis of triglucoside **14** in a capping cycle. a) 5 equiv **13**, 5 equiv TMSOTf, − 15 °C, CH_2Cl_2; b) A-Tag: 5 equiv **2**, DMAP, py/CH_2Cl_2; F-Tag: 5 equiv **11**, 2,6-lutidine; c) NH_2NH_2, 15 °C, py/AcOH; d) (1) ethylene, 0.2 equiv Grubbs' catalyst, CH_2Cl_2; (2) A-Tag: PBu_3/THF/water, then isocyanate silica gel scavenger resin; F-Tag: filtration through fluorous silica gel. Shaded circles = polystyrene polymer support. Lev = levulinate, Piv = pivaloyl, TMS = trimethylsilyl. Bottom: Synthesis of trimannoside **16** in a capping cycle. Coupling: 5 equiv **15**, 0.5 equiv TMSOTf, RT, CH_2Cl_2; F-Tag capping: 5 equiv **11**, 2,6-lutidine; deprotection: NaOMe, MeOH, RT; Cleavage: ethylene, 0.2 equiv Grubbs' catalyst, CH_2Cl_2; purification: filtration through fluorous silica gel.

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mixture obtained when no cap was used. Significant amounts of mono- and disaccharide deletion sequences were observed close to the desired triglucoside **14** in the synthesis without cap-tag (Figure 1a). These impurities could be almost completely removed by using the A-Tag, which greatly facilitated the purification process (Figure 1b). The F-Tag was similarly effective in the synthesis of **14** when the crude product was filtered through a plug of fluorous silica gel after cleavage from the solid support (Figure 1c).

The efficacy of the F-Tag strategy was also demonstrated for couplings involving secondary hydroxyl acceptors by incorporating a capping step into the solid-phase assembly of trimannoside **16** (Scheme 3 bottom).[13] The disappearance of side products and a significantly improved purity of the crude products can be clearly seen on comparing HPLC and TLC analyses of syntheses performed with and without the F-Tag (Figure 2). The HPLC traces of the crude products clearly reveal the efficiency of the F-Tag in removing unglycosylated C2-hydroxyl-containing deletion sequences (Figure 2a, b). Note that the simultaneous preparation of different oligosaccharide sequences that can be discriminated by introduction of unique F-Tags (containing varying numbers of fluorine atoms) at each stage of the synthesis is within reach.

In summary, we have introduced a new approach to facilitate the purification of oligosaccharides prepared by automated solid-phase synthesis. Incorporation of a capping step allowed for efficient removal of the unwanted side products at the end of the synthesis by use of a scavenger resin or filtration through fluorinated silica gel. The cap-tag method allows oligosaccharides to be obtained without the need for

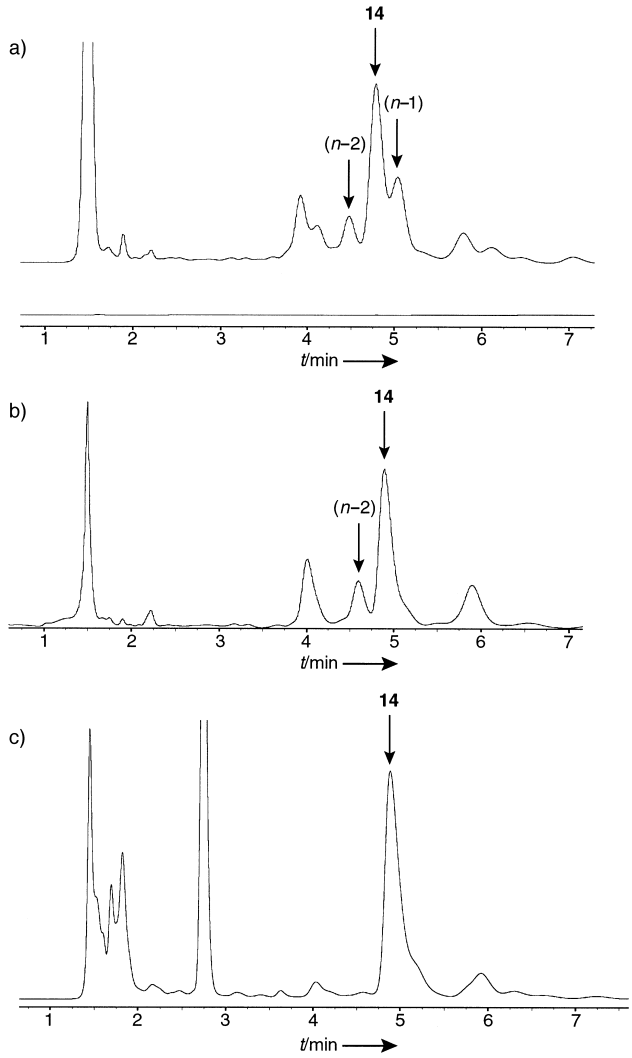
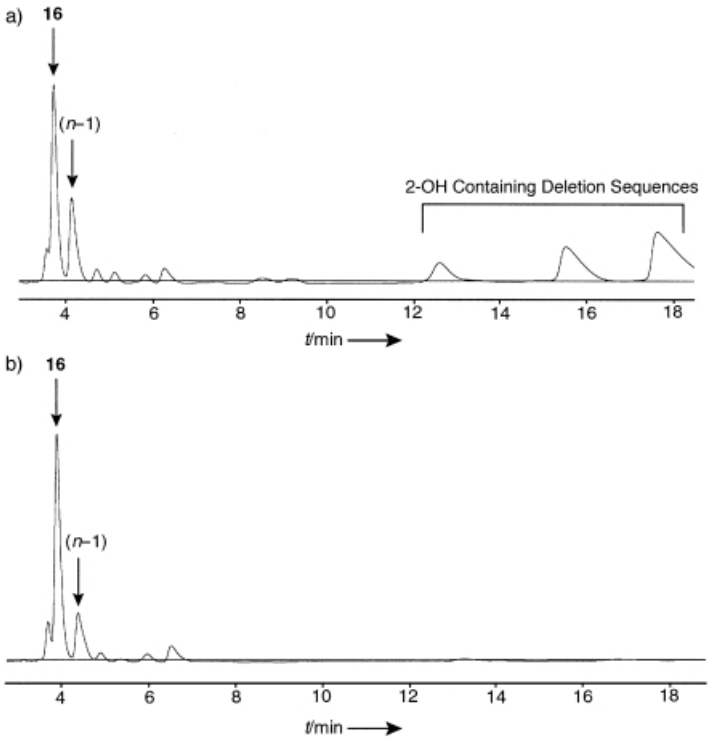


Figure 1. Analytical HPLC of triglucoside **14** syntheses. a) Synthesis without cap-tag; b) synthesis with A-Tag; c) synthesis with F-Tag. The indentity of the unmarked peaks could not be determined. Conditions: 1 mL min^{-1}, 20% EtOAc/hexanes.



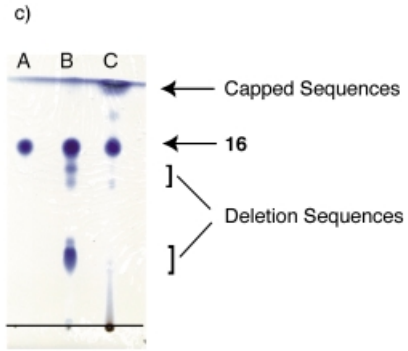


Figure 2. Analytical HPLC of trimannoside **16** syntheses a) without cap-tag (deletion sequences with C2 acetate groups are eluted at about 4 min, and those with C2 hydroxyl groups after 12 min), b) with F-Tag. Conditions: 1 mL min^{-1}, 20% EtOAc/hexanes. c) TLC analysis of trimannoside **16** syntheses. Lane A: pure **16;** lane B: crude **16** prepared without cap; lane C: crude **16** prepared with F-cap. Conditions: 30% EtOAc/hexanes.

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double couplings and a large excess of building blocks. The application of the A-Tag and the F-Tag to the automated synthesis of more complex oligosaccharides and other biopolymers is currently being explored.

Experimental Section

General Procedure for the automated synthesis of oligosaccharides incorporating cap-tags: Octenediol-functionalized resin **12** was loaded into a reaction vessel equipped with a cooling jacket and inserted into a modified ABI-433A peptide synthesizer. The resin was glycosylated with donor **13** or **15** (5 equiv) in CH_2Cl_2 (3 mL) with TMSOTf as activator. The suspension was mixed (10 s vortex, 50 s rest) for 15 min. The resin was then washed with CH_2Cl_2 (6×4 mL), and the unglycosylated sites were capped with A-Tag anhydride **2** or F-Tag triflate **11**. The resin was subjected to the appropriate deprotection conditions followed by the washing cycle. The deprotected polymer-bound monosaccharide was then elongated by reiteration of the above glycosylation/capping/deprotection protocol. The final trisaccharide was not deprotected, so that analysis of the products was simplified. For A-Tag, the crude material was treated with tributylphosphine and water, liberated from the resin, then purified with an isocyanate scavenger resin. For F-Tag, the crude material was liberated from the resin and purified with fluorous silica gel.

Received: August 2, 2001 [Z17654]

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From CO_2 to Methanol by Hybrid QM/MM Embedding**

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For improvements to be made in long-standing industrial catalytic processes, an understanding of the atomistic mechanisms of the reactions occurring on the surface of the catalyst is required. A variety of experimental techniques can be used to derive information on sorption and reaction processes, but when both the catalyst and reactant mixtures are multicomponent, unambiguous identification of reaction mechanisms is difficult and often controversial. Computational techniques can, however, be used to gain valuable insight and interpret experimental evidence. Herein we show how new methods for modeling surface reactions on oxides can be used to elucidate key steps in a widely studied catalytic process—the conversion of CO_2 to methanol over oxide catalysts.

A large quantity of methanol (in excess of 25 million tonnes worldwide) is produced annually using the multicomponent $Cu/ZnO/Al_2O_3$ catalyst and $CO_2/CO/H_2$ as the feed gas. Many experimental studies of this process have been performed, but no definite reaction mechanism for the production of methanol has been established. However, it has long been acknowledged that the important rate-determining step is the hydrogenation of adsorbed intermediates, for example, the formate ion, at the active sites. Proposed mechanisms for methanol synthesis require the chemisorption of CO_2 before hydrogenation via formate to methanol. Theoretical studies of these systems have been hampered by the difficulty of modeling the catalytically active polar surfaces of ZnO, as well as by problems associated with the restrictions on the size of the system that can be modeled.

The nature of the active site for sorption/catalysis of CO_2 still remains unclear; it has been proposed to use clean oxygen-terminated surfaces of zincite as a test system or model catalyst. Temperature-programmed desorption (TPD) studies have shown that the processes that occur at that

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[**] This work was supported by EU Esprit IV project 25047. S.A.F. is grateful to ICI and Synetix for funding. K. Waugh, L. Whitmore, S. Cristol, and P. Sushko are thanked for their helpful insights. QM/MM = quantum mechanics/molecular mechanics.

  1433-7851/01/4023-4437 $ 17.50+.50/0