the 4′-*O*-β-D-xylopyranosyl paromomycin showed antibacterial ribosomal activity comparable with that of paromomycin, but was significantly more selective with reduced affinity for the cytosolic ribosome and drug-induced ototoxicity. Importantly, the 4′-*O*-glycosylated paromomycin derivatives were not susceptible to modification by ANT(4′,4″) AME.

These approaches highlighted that single synergistic modifications are promising strategies to be used in new generations of AGAs to reduce toxicity and overcome resistance mechanisms.

1.3.2 Aminoglycoside Microarrays to Screen Interactions of Antibiotics with RNAs and Proteins

The study of protein–carbohydrate molecular interactions has been revolutionized by the advent of the high-throughput carbohydrate microarray technology (Rillahan and Paulson 2011; Palma et al. 2014). Carbohydrate microarrays are composed of diverse carbohydrate probe libraries printed onto a microarray solid surface as spatially oriented microspots. This miniaturization feature of the microarrays enables the screening of a wide range of interactions and generation of a large amount of information on different recognition systems. In addition, the multivalent display of arrayed probes enables the microarray to detect with high-sensitivity carbohydrate–protein interactions.

Early on the advent of carbohydrate microarray technology, Seeberger and colleagues (Disney and Seeberger 2004; Disney et al. 2004) adapted the chemistry of preparing carbohydrate microarrays to covalently immobilize unmodified aminoglycosides, through their amino groups, onto functionalized surfaces, such as *N*-hydroxysuccinimide (NHS)-activated glass slides, through an amide bond, or aldehyde-coated glass slides, through a carbon–nitrogen double bond (Figure 1.4a). In this proof-of-concept study, the authors proved the potential of the microarrays derived from 4,5- and 4,6-linked 2-DOS derivatives to screen interactions with mimics of aminoglycoside binding sites in the ribosome (bacterial and human rRNA A-sites) and with model proteins to study aminoglycoside toxicity (DNA polymerase and phospholipase C). These studies opened new avenues for the rapid screening and identification of new compounds that bind RNA tightly and exhibit decreased affinity toward resistance- and toxicity-causing proteins (Barrett et al. 2008; Disney et al. 2008).

Although attractive, a major limitation of these microarray strategies was the nonspecific way the aminoglycosides were immobilized onto the surface, which could lead to erroneous interpretation of the binding affinities (Disney and Seeberger 2004). New libraries immobilized through functional groups not predicted to interact with the target were an important development (Disney and Childs-Disney 2007; Barrett et al. 2008; Disney 2012). For example, Disney and colleagues (Barrett et al. 2008) modified aminoglycosides with an azido group at the hydroxyl group, enabling immobilization onto alkyne-displaying